STIC-Biot ch/ChemLib 45829

From: Chan, Christina
Sent: Monday, July 02, 2001 5:37 PM
To: Minnifield, Nita; STIC-Biotech/ChemLib
Subject: RE: rush interference seqeunce search

Please rush. Thanks Chris

Chris Chan
TC 1600 New Hire Training Coordinator and SPE, 1644
CM 1, Room 9B19
308-3973

-----Original Message-----
From: Minnifield, Nita
Sent: Monday, July 02, 2001 4:45 PM
To: Chan, Christina
Subject: rush interference seqeunce search

Christina,
please approve, 2 month amdt

Edward Hart
Technical Info Specialist
STIC / Biotech
CM1 12C14 Tel: 305-9203

STIC

09/117447

Please do a commercial and interference sequence search on SEQ ID NO: 1 of the above application.

Please provide a paper copy of the results.

THANKS!!!

Exr. N. M. Minnifield
AU 1645
CM1-8A07
Mailbox CM1-8E12
703-305-3394

9. A fusion protein comprising at least one polypeptide according to any one of claims 7 and 8.

9/117447

7/01

=> e lubitz werner/au

E1 610 LUBITZ W/AU
E2 4 LUBITZ W */AU
E3 134 --> LUBITZ WERNER/AU
E4 88 LUBITZ WOLFGANG/AU
E5 1 LUBITZKI LOTHAR/AU
E6 5 LUBITZSCH P/AU
E7 7 LUBITZSCH PETER/AU
E8 1 LUBITZSCH R/AU
E9 6 LUBITZSCH W/AU
E10 6 LUBITZSCH WOLFGANG/AU
E11 1 LUBIVYI V G/AU
E12 1 LUBIZ M/AU

=> s e1-e3

L18 748 ("LUBITZ W"/AU OR "LUBITZ W *"/AU OR "LUBITZ WERNER"/AU)

=> e sleytr uwe/au

E1 533 SLEYTR U B/AU
E2 1 SLEYTR UVE B/AU
E3 8 --> SLEYTR UWE/AU
E4 197 SLEYTR UWE B/AU
E5 15 SLEYTR UWE BERND/AU
E6 1 SLEYTR UWE R/AU
E7 1 SLEYTRE B/AU
E8 1 SLEZ AK J/AU
E9 1 SLEZ AK K/AU
E10 10 SLEZ AK P/AU
E11 1 SLEZ L/AU
E12 13 SLEZ L G/AU

=> s e1-e6

L19 752 ("SLEYTR U B"/AU OR "SLEYTR UVE B"/AU OR "SLEYTR UWE"/AU OR "SLEYTR UWE B"/AU OR "SLEYTR UWE BERND"/AU OR "SLEYTR UWE R"/AU)

=> e kuen beatrix/au

E1 1 KUEN B S/AU
E2 1 KUEN BEA/AU
E3 25 --> KUEN BEATRIX/AU
E4 2 KUEN C/AU
E5 1 KUEN C F/AU
E6 2 KUEN C K/AU
E7 2 KUEN C L/AU
E8 1 KUEN C Y/AU

E9 1 KUEN CHAN LAU/AU
E10 2 KUEN CHI FOUN/AU
E11 1 KUEN CHOY K/AU
E12 3 KUEN D/AU

=> s e1-e3

L20 27 ("KUEN B S"/AU OR "KUEN BEA"/AU OR "KUEN BEATRIX"/AU)

=> e truppe michaela/au

E1 5 TRUPPE MICHAEL/AU
E2 2 TRUPPE MICHAEL J/AU
E3 5 --> TRUPPE MICHAELA/AU
E4 1 TRUPPE MICHAELA SONJA/AU
E5 2 TRUPPE P/AU
E6 1 TRUPPE PETER/AU
E7 1 TRUPPE R B/AU
E8 1 TRUPPE ROBERT B/AU
E9 1 TRUPPE ROBERT E/AU
E10 2 TRUPPE S/AU
E11 53 TRUPPE W/AU
E12 8 TRUPPE WOLFGANG/AU

=> s e3-e4

L21 6 ("TRUPPE MICHAELA"/AU OR "TRUPPE MICHAELA SONJA"/AU)

=> e howorka stefan/au

E1 23 HOWORKA S/AU
E2 1 HOWORKA SIEGFRIED/AU
E3 12 --> HOWORKA STEFAN/AU
E4 2 HOWORKA STEPHAN/AU
E5 1 HOWORKO ADOLF C/AU
E6 1 HOWORKO N/AU
E7 1 HOWORT P/AU
E8 9 HOWORTH A/AU
E9 14 HOWORTH A J/AU
E10 1 HOWORTH ALISON/AU
E11 2 HOWORTH ALISON J/AU
E12 28 HOWORTH B/AU

=> s e1-e4

L22 38 ("HOWORKA S"/AU OR "HOWORKA SIEGFRIED"/AU OR "HOWORKA STEFAN"/AU
OR "HOWORKA STEPHAN"/AU)

=> e resch stepanka/au

E1 6 RESCH STEFAN/AU
E2 1 RESCH STEFANIE/AU
E3 1 --> RESCH STEPANKA/AU
E4 4 RESCH STEPHANIE/AU
E5 1 RESCH STEPHNIE/AU

E6 1 RESCH STEVEN C/AU
E7 3 RESCH SYLVIA/AU
E8 37 RESCH T/AU
E9 1 RESCH T A/AU
E10 1 RESCH T J/AU
E11 1 RESCH T L/AU
E12 1 RESCH TH/AU

=> s e1-e5

L23 13 ("RESCH STEFAN"/AU OR "RESCH STEFANIE"/AU OR "RESCH STEPANKA"/AU OR "RESCH STEPHANIE"/AU OR "RESCH STEPHNIE"/AU)

=> e schroll gerhard/au

E1 14 SCHROLL GENE E/AU
E2 2 SCHROLL GEORG/AU
E3 8 --> SCHROLL GERHARD/AU
E4 2 SCHROLL GOTTFRIED/AU
E5 4 SCHROLL GUENTER/AU
E6 47 SCHROLL GUSTAV/AU
E7 26 SCHROLL H/AU
E8 1 SCHROLL HANS/AU
E9 2 SCHROLL HARSTED BJARNE/AU
E10 2 SCHROLL HENNING/AU
E11 7 SCHROLL J/AU
E12 1 SCHROLL J T/AU

=> s e3

L24 8 "SCHROLL GERHARD"/AU

=> e sara margit/au

E1 4 SARA MAAGARETSUTO JIENKINZU/AU
E2 1 SARA MAKUROFU/AU
E3 125 --> SARA MARGIT/AU
E4 3 SARA MARTIN N/AU
E5 8 SARA MAURIZIO/AU
E6 1 SARA MAUSHIYU/AU
E7 1 SARA MEESON RITSUCHIMONDO/AU
E8 1 SARA MESHIERI/AU
E9 1 SARA MEZA C R/AU
E10 35 SARA MICHELE/AU
E11 1 SARA MIRUDORETSUDO BISHIYOTSUPU/AU
E12 1 SARA MOUNA/AU

=> s e3

L25 125 "SARA MARGIT"/AU

=> s l18-l25

L26 1524 (L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25)

=> s l26 and (s layer protein or s-layer protein)

6 FILES SEARCHED...
L27 269 L26 AND (S LAYER PROTEIN OR S-LAYER PROTEIN)

=> s l27 and (recombinant or fusion)

L28 31 L27 AND (RECOMBINANT OR FUSION)

=> dup rem l28

PROCESSING COMPLETED FOR L28
L29 15 DUP REM L28 (16 DUPLICATES REMOVED)

=> d bib ab 1-15

L29 ANSWER 1 OF 15 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 1
AN 2001183818 EMBASE
TI Analysis of the structure-function relationship of the ***S*** - ***layer*** ***protein*** SbsC of Bacillus stearothermophilus ATCC 12980 by producing truncated forms.
AU Jarosch M.; Egelseer E.M.; Huber C.; Moll D.; Mattanovich D.; ***Sleytr*** *** U.B.*** ; Sara M.
CS M. Sara, Ctr. for Ultrastructure Res./Ludwig, Boltzmann-Inst. Mol. Nanotec., University of Agricultural Sciences, 1180 Vienna, Austria. sara@edv1.boku.ac.at
SO Microbiology, (2001) 147/5 (1353-1363).
Refs: 31
ISSN: 1350-0872 CODEN: MROBEO
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The mature surface layer (***S*** - ***layer***) ***protein*** SbsC of Bacillus stearothermophilus ATCC 12980 comprises amino acids 31-1099 and self-assembles into an oblique lattice type which functions as an adhesion site for a cell-associated high-molecular-mass exoamylase. To elucidate the structure-function relationship of distinct segments of SbsC, three N- and seven C-terminal truncations were produced in a heterologous expression system, isolated, purified and their properties compared with those of the ***recombinant*** mature ***S*** - ***layer*** ***protein*** rSbsC(31-1099). With the various truncated forms it could be demonstrated that the N-terminal part (aa 31-257) is responsible for anchoring the S-layer subunits via a distinct type of secondary cell wall polymer to the rigid cell wall layer, but this positively charged segment is not required for the self-assembly of SbsC, nor for generating the oblique lattice structure. If present, the N-terminal part leads to the formation of in vitro double-layer self-assembly products. Affinity studies further showed that the N-terminal part includes an exoamylase-binding site. Interestingly, the N-terminal part carries two sequences of 6 and 7 aa (AKAALD and KAAYEAA) that were also identified on the amylase-binding protein AbpA of Streptococcus gordonii. In contrast to the self-assembling N-terminal

truncation rSbsC(258-1099), two further N-terminal truncations (rSbsC(343-1099), rSbsC(447-1099)) and three C-terminal truncations (rSbsC(31-713), rSbsC(31-844), rSbsC(31-860)) had lost the ability to self-assemble and stayed in the water-soluble state. Studies with the self-assembling C-terminal truncations rSbsC(31-880), rSbsC(31-900) and rSbsC(31-920) revealed that the C-terminal 219 aa can be deleted without interfering with the self-assembly process, while the C-terminal 179 aa are not required for the formation of the oblique lattice structure.

L29 ANSWER 2 OF 15 BIOSIS COPYRIGHT 2001 BIOSIS
AN 2000:439659 BIOSIS
DN PREV200000439659
TI ISBst12, a novel type of insertion-sequence element causing loss of S-layer-gene expression in Bacillus stearothermophilus ATCC 12980.
AU Egelseer, Eva M. (1); Idris, Rughia; Jarosch, Marina; Danhorn, Thomas; ***Sleytr, Uwe B.*** ; ***Sara, Margit***
CS (1) Zentrum fuer Ultrastrukturforschung und Ludwig Bolzmann, Institut fuer Molekulare Nanotechnologie, Universitaet fuer Bodenkultur, A-1180, Vienna Austria
SO Microbiology (Reading), (September, 2000) Vol. 146, No. 9, pp. 2175-2183. print.
ISSN: 1350-0872.
DT Article
LA English
SL English
AB The cell surface of the surface layer (S-layer)-carrying strain of Bacillus stearothermophilus ATCC 12980 is completely covered with an oblique lattice composed of the ***S*** - ***layer*** ***protein*** SbsC. In the S-layer-deficient strain, the S-layer gene sbsC was still present but was interrupted by a novel type of insertion sequence (IS) element designated ISBst12. The insertion site was found to be located within the coding region of the sbsC gene, 199 bp downstream from the translation start of SbsC. ISBst12 is 1612 bp long, bounded by 16 bp imperfect inverted repeats and flanked by a directly repeated 8 bp target sequence. ISBst12 contains an ORF of 1446 bp and is predicted to encode a putative transposase of 482 aa with a calculated theoretical molecular mass of 55562 Da and an isoelectric point of 9.13. The putative transposase does not exhibit a typical DDE motif but displays a His-Arg-Tyr triad characteristic of the active site of integrases from the bacteriophage lambda Int family. Furthermore, two overlapping leucine-zipper motifs were identified at the N-terminal part of the putative transposase. As revealed by Southern blotting, ISBst12 was present in multiple copies in the Slayer-deficient strain as well as in the S-layer-carrying strain. Northern blotting indicated that S-layer gene expression is already inhibited at the transcriptional level, since no sbsC-specific transcript could be identified in the S-layer-deficient strain. By using PCR, ISBst12 was also detected in B. stearothermophilus PV72/p6, in its oxygen-induced strain variant PV72/p2 and in the S-layer-deficient strain PV72/T5.

L29 ANSWER 3 OF 15 MEDLINE DUPLICATE 2
AN 2000170659 MEDLINE
DN 20170659 PubMed ID: 10708365
TI S-layer gene sbsC of Bacillus stearothermophilus ATCC 12980: molecular characterization and heterologous expression in Escherichia coli.

AU Jarosch M; Egelseer E M; Mattanovich D; ***Sleytr U B*** ; Sara M
CS Zentrum fur Ultrastrukturforschung und Ludwig Boltzmann-Institut fur Molekulare Nanotechnologie, Universitat fur Bodenkultur, Vienna, Austria.
SO MICROBIOLOGY, (2000 Feb) 146 (Pt 2) 273-81.
Journal code: BXW; 9430468. ISSN: 1350-0872.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AF055578
EM 200005
ED Entered STN: 20000512
Last Updated on STN: 20000512
Entered Medline: 20000501
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered with an oblique S-layer lattice. To investigate sequence identities and a common structure-function relationship in S-layer proteins of different B. stearothermophilus wild-type strains, the nucleotide sequence encoding the ***S*** - ***layer*** ***protein*** SbsC of B. stearothermophilus ATCC 12980 was determined by PCR techniques. The entire sbsC sequence showed an ORF of 3297 bp predicted to encode a protein of 1099 aa with a theoretical molecular mass of 115409 Da and an isoelectric point of 5.73. Primer extension analysis suggested the existence of two promoter regions. Amino acid sequence comparison between SbsC and SbsA, a previously characterized ***S*** - ***layer*** ***protein*** of B. stearothermophilus PV72/p6 which assembles into a hexagonally ordered lattice, revealed an identical secretion signal peptide, 85% identity for the N-terminal regions (aa 31-270) which do not carry any S-layer homologous motifs, but only 21% identity for the rest of the sequences. Affinity studies demonstrated that the N-terminal part of SbsC is necessary for recognition of a secondary cell wall polymer. This was in accordance with results obtained in a previous study for SbsA, thus confirming a common functional principle for the N-terminal parts of both S-layer proteins. The sbsC coding region cloned into the pET3a vector without its own upstream region, the signal sequence and the 3' transcriptional terminator led to stable expression in Escherichia coli.

L29 ANSWER 4 OF 15 BIOSIS COPYRIGHT 2001 BIOSIS
AN 2000:418709 BIOSIS
DN PREV200000418709
TI The transposable element IS4712 prevents S-layer gene (sbsA) expression in Bacillus stearothermophilus and also affects the synthesis of altered surface layer proteins.
AU Scholz, Holger (1); Hummel, Susanne; Witte, Angela; ***Lubitz, Werner*** ; ***Kuen, Beatrix***
CS (1) Institute of Animal Hygiene and Public Veterinary Health, An den Tierkliniken 43, 04103, Leipzig Germany
SO Archives of Microbiology, (July August, 2000) Vol. 174, No. 1-2, pp. 97-103. print.
ISSN: 0302-8933.
DT Article
LA English
SL English
AB Cell surface (***S***)- ***layer*** ***protein*** synthesis in

Bacillus stearothermophilus PV72/p6 is blocked when cells are grown at elevated temperature. From a culture exhibiting the S-layer-negative phenotype, the S-layer deficient mutant T5 (SbsA-) was isolated. Genetic analysis of the S-layer-encoding gene (sbsA) of mutant T5 revealed an insertion element (IS4712) integrated into the upstream regulatory region of the S-layer gene, thereby blocking sbsA transcription. The insertion element consists of 1371 base pairs which are flanked by two perfect inverted terminal repeats. Sequence similarity to other transposases of the IS4 family was detected. DNA-DNA hybridizations demonstrated that multiple homologues of IS4712 were also present within the genomes of several other thermophilic bacillus isolates. Attempts to isolate SbsA+ revertants failed. Instead, cells with altered surface proteins were detected. The synthesis of the altered S-layer proteins was correlated with the presence of IS4712 along with the occurrence of deletions in the sbsA coding region. Furthermore imprecise excision of IS4712 was detected. This work demonstrated that B. stearothermophilus is able to express at least four different S-layer proteins and that blocking of sbsA transcription by the insertion element IS4712 is associated with the expression of altered surface proteins.

L29 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3
AN 1999:96508 CAPLUS
DN 130:178339
TI Production of genetically engineered ***S*** - ***layer*** ***protein*** that is secreted into the periplasm or extracellularly and that can contain integrated proteins for affinity and immuno reactions
IN ***Lubitz, Werner*** ; ***Resch, Stephanie***
PA Austria
SO Ger. Offen., 34 pp.
CODEN: GWXXBX
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI DE 19732829	A1	19990204	DE 1997-19732829	19970730
WO 9906567	A1	19990211	WO 1998-EP4723	19980727

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9890705	A1	19990222	AU 1998-90705	19980727
EP 1005553	A1	20000607	EP 1998-942648	19980727

R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE, IE

PRAI DE 1997-19732829 19970730
WO 1998-EP4723 19980727
AB The invention concerns the prodn. of ***recombinant*** ***S*** - ***layer*** ***protein*** expressed in Gram-neg. prokaryote or eukaryote host cells using the sbsA and sbsB clones of the Bacillus stearothermophilus PV72, that code for the ***S*** - ***layer*** ***protein*** and the prokaryote signal peptide; the vector also

contains inserts at convenient sites that code for various peptides, e.g. cysteine residues, DNA-binding epitopes, metal-binding epitopes, allergens, antigens, streptavidin, enzymes etc. In case the ***fusion*** protein is expressed in eukaryotes, the vector includes sequences coding for eukaryote signal peptides. The host cell contains at least two types of genes that code for the a non-modified ***S*** - ***layer*** ***protein*** and for a modified ***S*** - ***layer*** ***protein*** that is fused with a peptide used biochem. reactions. E.coli is a typical host cell.

L29 ANSWER 6 OF 15 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1999-11466 BIOTECHDS
TI Extended ***recombinant*** bacterial ghost system; ghost cell production and foreign gene and antigen expression for use as a ***recombinant*** combination vaccine (conference paper)
AU ***Lubitz W*** ; Witte A; Eko F O; Kamal M; Jechlinger W; Brand E; Marchart J; Haidinger W; Huter V; Felnerova D; Stralis-Alves N; Lechleitner S; Melzer H; Szostak M P; Resch S; Nader H; Kuen B; Mayr B; Mayrhofer P; Geretschlager R; Haslberger A; Hensel A
CS Univ.Vienna-Inst.Microbiol.Genet.; EVAX-Technol.; Univ.Leipzig-Inst.Anim.Hyg.Vet.Public-Health
LO Institute of Microbiology and Genetics, University of Vienna, Dr. Bohrgasse 9, A-1030 Vienna, Austria.
Email: oldfox@gem.univie.ac.at
SO J.Biotechnol.; (1999) 73, 2-3, 261-73
CODEN: JBITD4 ISSN: 0168-1656
New Approaches in Vaccine Development 1997, Australian Society of Biotechnology, Vienna, Austria, 1997.
DT Journal
LA English
AB Controlled expression of cloned PhiX174 gene E in Gram-negative bacteria results in lysis of the bacteria by formation of an E-specific transmembrane tunnel structure built through the cell envelope complex. These bacterial ghosts from a variety of bacteria were used as non-living candidate vaccines. In a ***recombinant*** ghost system, the desired foreign proteins are attached to the inside of the inner membrane as fusions with specific anchor sequences. Because the ghosts have a sealed periplasmic and the proteins can be exported into this space the capacity of the ghost or ***recombinant*** ghost systems can be vastly extended, therefore making them capable carriers of foreign antigens. The ***recombinant*** ghosts can also express ***S*** - ***layer*** ***protein*** (shell-like structure), which can carry foreign gene epitopes, which further extends the possibilities of ghost carriers. The ghost also have inherent adjuvant properties, so they can be used as adjuvants in combination with subunit vaccines. There is no limitations on the size of foreign antigens which can be inserted into the ghosts and so they may be used as adjuvant free combination vaccines. (32 ref)

L29 ANSWER 7 OF 15 MEDLINE DUPLICATE 4
AN 1999204063 MEDLINE
DN 99204063 PubMed ID: 10188248
TI Self-assembly product formation of the Bacillus stearothermophilus PV72/p6 ***S*** - ***layer*** ***protein*** SbsA in the course of autolysis of Bacillus subtilis.
AU ***Howorka S*** ; Sara M; ***Lubitz W*** ; Kuen B

CS Institut fur Mikrobiologie and Genetik, Universitat Wien, Vienna, Austria.
SO FEMS MICROBIOLOGY LETTERS, (1999 Mar 15) 172 (2) 187-96.
Journal code: FML; 7705721. ISSN: 0378-1097.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199904
ED Entered STN: 19990511
Last Updated on STN: 19990511
Entered Medline: 19990429
AB In order to achieve high level expression and to study the release of a protein capable of self-assembly, the gene encoding the crystalline cell surface (***S*** - ***layer***) ***protein*** SbsA of Bacillus stearothermophilus PV72/p6, including its signal sequence, was cloned and expressed in Bacillus subtilis. To obtain high level expression, a tightly regulated, xylose-inducible, stably replicating multicopy-plasmid vector was constructed. After induction of expression, the ***S*** - ***layer*** ***protein*** made up about 15% of the total cellular protein content, which was comparable to the SbsA content of B. stearothermophilus PV72/p6 cells. During all growth stages, SbsA was poorly secreted to the ambient cellular environment by B. subtilis. Extraction of whole cells with guanidine hydrochloride showed that in late stationary growth phase cells 65% of the synthesised SbsA was retained in the peptidoglycan-containing layer, indicating that the rigid cell wall layer was a barrier for efficient SbsA secretion. Electron microscopic investigation revealed that SbsA release from the peptidoglycan-containing layer started in the late stationary growth phase at distinct sites at the cell surface leading to the formation of extracellular self-assembly products which did not adhere to the cell wall surface. In addition, intracellular sheet-like SbsA self-assembly products which followed the curvature of the cell became visible in partly lysed cells. Intracellularly formed self-assembly products remained intact even after complete lysis of the rigid cell envelope layer.

L29 ANSWER 8 OF 15 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 5
AN 1998274055 EMBASE
TI A novel dipstick developed for rapid bet v 1-specific IgE detection: ***Recombinant*** allergen immobilized via a monoclonal antibody to crystalline bacterial cell-surface layers.
AU Breitwieser A.; Mader C.; Schocher I.; Hoffmann-Sommergruber K.; Aberer W.; Scheiner O.; ***Sleytr U.B.*** ; Sara M.
CS Prof. M. Sara, Zentrum fur Ultrastrukturforschung, Universitat fur Bodenkultur, Gregor-Mendelstr. 33, 1180 Wien, Austria
SO Allergy: European Journal of Allergy and Clinical Immunology, (1998) 53/8 (786-793).
Refs: 25
ISSN: 0105-4538 CODEN: LLRGDY
CY Denmark
DT Journal; Article
FS 026 Immunology, Serology and Transplantation
LA English
SL English
AB The incidence of allergy to airborne proteins derived from tree and grass pollen, feces of mites, spores of molds, and pet dander has been

increasing over the last decades. Since precise diagnosis is a prerequisite for successful immunotherapy, there is a rising demand for rapid, reliable, and inexpensive screening methods such as dipstick assays. With the purified ***recombinant*** major birch-pollen allergen rBet v 1a as model protein, crystalline bacterial cell-surface layers (S-layers) were tested for their applicability as an immobilization matrix for dipstick development. For this purpose, S-layers were deposited on a mechanically stable microporous support, cross-linked with glutaraldehyde, and free carboxylic acid groups of the ***S*** - ***layer*** ***protein*** were activated with carbodiimide. In the present test system, rBet v 1a was immobilized via the monoclonal mouse antibody BIP 1, which, unlike the allergen, is too large to enter the pores of the S-layer lattice, and which therefore formed a closed monolayer on the outermost surface of the crystal lattice. Moreover, BIP 1 is known to modulate IgE binding to the allergen. After incubation of the dipsticks in serum, washing of the reaction zone under tap water, and binding of an anti-IgE alkaline phosphatase conjugate, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium was used as substrate, forming an IgE concentration-dependent colored precipitate on the S-layer surface. The investigation of patient sera previously tested with the CAP(TM) system confirmed the specificity of the S- layer-based dipstick assay. Since the dipstick is easy to handle and the whole test procedure takes only 90 min, this test system should be applicable for rapid determination of specific IgE and for first screening in the doctor's practice.

L29 ANSWER 9 OF 15 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD DUPLICATE 6
AN 1997-394558 [37] WPIDS
DNC C1997-126945
TI Preparation of S-layer proteins by expressing sbs-A gene in Gram negative bacterium - or new sbs-B gene in any host, also new ***recombinant*** proteins containing heterologous inserts, e.g. epitope(s), useful as vaccines and adjuvants.
DC B04 C06 D16
IN KUEN, B; ***LUBITZ, W*** ; SLEYTR, U; ***HOWORKA, S*** ; RESCH, S; SARA, M; SCHROLL, G; TRUPPE, M
PA (LUBI-I) LUBITZ W; (SLEY-I) SLEYTR U; (SLEY-I) SLEYTR U B
CYC 75
PI DE 19603649 A1 19970807 (199737)* 31p
WO 9728263 A1 19970807 (199737) DE 69p
RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN
AU 9717203 A 19970822 (199801)
EP 882129 A1 19981209 (199902) DE
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
NZ 331300 A 19990629 (199931)
CN 1213402 A 19990407 (199932)
AU 713999 B 19991216 (200010)
JP 2000503850 W 20000404 (200027) 61p
ADT DE 19603649 A1 DE 1996-19603649 19960201; WO 9728263 A1 WO 1997-EP432 19970131; AU 9717203 A AU 1997-17203 19970131, WO 1997-EP432 19970131; EP

882129 A1 EP 1997-904360 19970131, WO 1997-EP432 19970131; NZ 331300 A NZ 1997-331300 19970131, WO 1997-EP432 19970131; CN 1213402 A CN 1997-192940 19970131; AU 713999 B AU 1997-17203 19970131; JP 2000503850 W JP 1997-527307 19970131, WO 1997-EP432 19970131

FDT AU 9717203 A Based on WO 9728263; EP 882129 A1 Based on WO 9728263; NZ 331300 A Based on WO 9728263; AU 713999 B Previous Publ. AU 9717203, Based on WO 9728263; JP 2000503850 W Based on WO 9728263

PRAI DE 1996-19603649 19960201

AB DE 19603649 A UPAB: 19970926

Preparation of ***S*** - ***layer*** ***protein*** (I), comprises transforming a Gram-negative prokaryotic host with a (I) encoding nucleic acid (II), and culturing the transformed cells.

USE - (I) and the S-layer structures are useful as vaccines or adjuvants, particularly when they include a bacterial ghost that may contain additional epitopes in its membrane. Other uses of ***recombinant*** (I), depending in the nature of the inserted peptide, are as an universal carrier for biotinylated reactants for use in immunological or hybridisation assays (the insert is streptavidin), to induce immune responses (epitopes), as a reagent for removing cytokine or toxin from serum (antigenic epitopes), as a molecular spinning nozzle (polyhydroxybutyrate synthase, PHBS) and as molecular laser (luciferase).

ADVANTAGE - When expressed in Gram-negative cells, (I) are produced in the form of monomolecular layers, rather than as inclusion bodies as in Gram-positive bacteria.

Dwg.2/3

L29 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2001 ACS

AN 1997:536912 CAPLUS

DN 127:201021

TI Expression of S-layer proteins in Gram-negative bacteria and ***recombinant*** chimeric S-layer proteins for use as vaccines

IN ***Lubitz, Werner*** ; ***Sleytr, Uwe*** ; ***Kuen, Beatrix*** ; ***Truppe, Michaela*** ; ***Howorka, Stefan*** ; ***Resch,*** *** Stepanka*** ; ***Schroll, Gerhard*** ; ***Sara, Margit***

PA Lubitz, Werner, Austria; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit

SO PCT Int. Appl., 65 pp.
CODEN: PIXXD2

DT Patent

LA German

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9728263	A1	19970807	WO 1997-EP432	19970131
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
DE 19603649	A1	19970807	DE 1996-19603649	19960201
CA 2245584	AA	19970807	CA 1997-2245584	19970131
AU 9717203	A1	19970822	AU 1997-17203	19970131

AU 713999 B2 19991216
EP 882129 A1 19981209 EP 1997-904360 19970131
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
CN 1213402 A 19990407 CN 1997-192940 19970131
JP 2000503850 T2 20000404 JP 1997-527307 19970131
PRAI DE 1996-19603649 19960201
WO 1997-EP432 19970131

AB The invention concerns processess for the ***recombinant*** prepn. of S-layer proteins in Gram-neg. host cells. In addn., the nucleotide sequence of a new S-layer gene, the sbsB gene of Bacillus stearothermophilus, and a process for prepn. of modified S-layer proteins is disclosed. ***Recombinant*** Escherichia coli expressing the sbsA gene of B. stearothermophilus and chimeric sbsA genes encoding SbsA into which various peptides, proteins and enzymes have been inserted were prepd. and cultured to produce the proteins.

L29 ANSWER 11 OF 15 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1998:87452 BIOSIS
DN PREV199800087452
TI Bet v 1, the major birch pollen allergen, conjugated to crystalline bacterial cell surface proteins, expands allergen-specific T cells of the Th1/Th0 phenotype in vitro by induction of IL-12.
AU Jahn-Schmid, Beatrice; Siemann, Ute; Zenker, Andrea; Bohle, Barbara; Messner, Paul; Unger, Frank M.; ***Sleytr, Uwe B.*** ; Scheiner, Otto; Kraft, Dietrich; Ebner, Christof (1)
CS (1) Inst. Allgemeine Experimentelle Pathologie, Univ. Wien, AKH-EWB-OST 3Q, Waehringer Guertel 18-20, 1090 Wien Austria
SO International Immunology, (Dec., 1997) Vol. 9, No. 12, pp. 1867-1874. ISSN: 0953-8178.
DT Article
LA English
AB Modulation of allergic immune responses by using adequate adjuvants is a promising concept for future immunotherapy of type I hypersensitivity. In the present study, ***recombinant*** Bet v 1 (rBet v 1, the major birch pollen allergen) was conjugated to cross-linked crystalline surface layer proteins (SL) derived from Gram-positive eubacteria. T cell lines (TCL) and clones (TCC) were established from peripheral blood of birch pollen-allergic patients. TCL and TCC were induced either using rBet v 1 alone or rBet v 1/SL conjugates (rBet v 1/SL) as initial antigen stimulus. Cytokine production after re-stimulation with rBet v 1 was investigated. TCL initiated with rBet v 1/SL showed significantly increased IFN-gamma production as compared to rBet v 1-selected TCL. TCC were established from TCL of five patients. As expected, the majority of CD4+ TCC induced by rBet v 1 (55%) displayed a Th2-like pattern of cytokine production. However, only 21% of Bet v 1 -specific TCC isolated from TCL established with the Bet v 1/SL revealed this phenotype. The majority of SL-specific TCC (80%) belonged to the Th1 phenotype. In cultures of peripheral blood mononuclear cells, both, SL and Bet v 1/SL (but not rBet v 1) stimulated the production of high levels of IL-12, a pivotal mediator of Th1 responses. Moreover, stimulation of rBet v 1-induced TCC with rBet v 1/SL led to an Increased IFN-gamma production. This effect could be reversed by neutralizing anti-IL-12 mAb. Together these results indicate an adjuvant effect of SL mediated by IL-12. Our results indicate that bacterial components, such as SL, displaying adjuvant effects may be suitable for

immunotherapeutical vaccines for type I allergy.

L29 ANSWER 12 OF 15 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 7
AN 97267948 EMBASE
DN 1997267948
TI IV. Molecular biology of S-layers.
AU Bahl H.; Scholz H.; Bayan N.; Chami M.; Leblon G.; Gulik-Krzywicki T.; Shechter E.; Fouet A.; Mesnage S.; Tosi-Couture E.; Gounon P.; Mock M.; De Macario E.C.; Macario A.J.L.; Fernandez-Herrero L.A.; Olabarria G.; Berenguer J.; Blaser M.J.; Kuen B.; ***Lubitz W.*** ; Sara M.; Pouwels P.H.; Kolen C.P.A.M.; Boot H.J.; Palva A.; Truppe M.; ***Howorka S.*** ; Schroll G.; Lechleitner S.; Resch S.
CS Dr. N. Bayan, Laboratoire des Biomembranes, URA 1116 CNRS, Universite de Paris-Sud, F-91405 Orsay, France
SO FEMS Microbiology Reviews, (1997) 20/1-2 (47-98).
Refs: 197
ISSN: 0168-6445 CODEN: FMREE4
PUI S 0168-6445(97)00050-8
CY Netherlands
DT Journal; General Review
FS 004 Microbiology
LA English
SL English
AB In this chapter we report on the molecular biology of crystalline surface layers of different bacterial groups. The limited information indicates that there are many variations on a common theme. Sequence variety, antigenic diversity, gene expression, rearrangements, influence of environmental factors and applied aspects are addressed. There is considerable variety in the S-layer composition, which was elucidated by sequence analysis of the corresponding genes. In Corynebacterium glutamicum one major cell wall protein is responsible for the formation of a highly ordered, hexagonal array. In contrast, two abundant surface proteins form the S-layer of Bacillus anthracis. Each protein possesses three S-layer homology motifs and one protein could be a virulence factor. The antigenic diversity and ABC transporters are important features, which have been studied in methanogenic archaea. The expression of the S-layer components is controlled by three genes in the case of Thermus thermophilus. One has repressor activity on the S-layer gene promoter, the second codes for the ***S*** - ***layer*** ***protein*** . The rearrangement by reciprocal recombination was investigated in Campylobacter fetus. 7-8 S-layer proteins with a high degree of homology at the 5' and 3' ends were found. Environmental changes influence the surface properties of Bacillus stearothermophilus. Depending on oxygen supply, this species produces different S-layer proteins. Finaly, the molecular bases for some applications are discussed. ***Recombinant*** S-layer ***fusion*** proteins have been designed for biotechnology.

L29 ANSWER 13 OF 15 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD DUPLICATE 8
AN 1996-077933 [09] WPIDS
DNC C1996-025830
TI Nucleic acid encoding signal peptide of Bacillus stearothermophilus ***S*** - ***layer*** ***protein*** - which has a lysine content of at least 10 per cent..
DC B04 D16

IN ***LUBITZ, W***
PA (VOGE-N) VOGELBUSCH GMBH
CYC 1
PI DE 4425527 A1 19960125 (199609)* 11p
ADT DE 4425527 A1 DE 1994-4425527 19940719
PRAI DE 1994-4425527 19940719
AB DE 4425527 A UPAB: 19960305
Nucleic acid (I) that codes for a functional signal peptide (SP) is new which is selected from: (a) the SP-encoding portion of a 3706 bp sequence given in the specification; (b) a sequence corresp. to (a) taking into account the degeneracy of the genetic code, or (c) a sequence with at least 90 % homology to (a) or (b). Also claimed are: (1) (I) operatively linked at its 3' terminus to a protein-encoding nucleic acid; (2) (I) or the nucleic acid of (1) operatively linked at its 5' terminus to an expression control sequence; (3) a polypeptide encoded by a nucleic acid as above; (4) a ***recombinant*** vector contg. at least one copy of a nucleic acid as above; (5) a host cell transformed with a nucleic acid or vector as above; and (6) an expression control sequence selected from: (a) the nucleotide sequence of 247 bp as given in the specification; (b) a sequence corresp. to (a) taking into account the degeneracy of the genetic code; and (c) a sequence with at least 90 % homology to (a) or (b).
USE - The process is useful for prodn. of B. stearothermophilus ***S*** - ***layer*** ***protein*** , which has a lysine content of at least 10 %. Opt. the protein is hydrolysed and the amino acids recovered.
Dwg.0/1

L29 ANSWER 14 OF 15 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 9
AN 96346348 EMBASE
DN 1996346348
TI 2-D protein crystals as an immobilization matrix for producing reaction zones in dipstick-style immunoassays.
AU Breitwieser A.; Kupcu S.; ***Howorka S.*** ; Weiger S.; Langer C.; Hoffmann- Sommergruber K.; Scheiner O.; ***Sleytr U.B.*** ; Sara M.
CS ZULB, Inst. fur Molekulare Nanotechnologie, Universitat fur Bodenkultur, Gregor Mendelstrasse 33,A-1180 Vienna, Austria
SO BioTechniques, (1996) 21/5 (918-925).
ISSN: 0736-6205 CODEN: BTNQDO
CY United States
DT Journal; Article
FS 004 Microbiology
027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
LA English
SL English
AB In the present study, the applicability of crystalline bacterial cell-surface layers (S-layers) as novel immobilization matrices and reaction zones for dipstick-style immunoassays was investigated. For this purpose, S-layer- carrying cell-wall fragments from Bacillus sphaericus CCM 2120 were deposited on a microporous support, and the ***S*** - ***layer*** ***protein*** was cross-linked with glutaraldehyde. For developing appropriate test systems, either human IgG was directly linked to the carboxylic acid groups from the ***S*** - ***layer*** ***protein*** or it was immobilized using Protein A or, after biotinylation, using streptavidin. A clear correlation was obtained

between the amount of anti-human IgG applied and the absorbance values in the immunoassays. S-layers with covalently bound ***recombinant*** major birch pollen allergen were used for quantitative and semiquantitative determination of an antibody raised against it. Using S-layers as an immobilization matrix in comparison to amorphous polymers has advantages in that the closed monolayers of functional macromolecules on their outermost surface allows for strong signals in immunoassays, almost completely eliminates background and prevents diffusion.

L29 ANSWER 15 OF 15 MEDLINE
AN 96228698 MEDLINE
DN 96228698 PubMed ID: 8830240
TI Heterologous expression and self-assembly of the ***S*** - ***layer*** ***protein*** SbsA of Bacillus stearothermophilus in Escherichia coli.
AU Kuen B; Sara M; ***Lubitz W***
CS Institut fur Mikrobiologie und Genetik, Universitat, Austria.. oetzi@gem.univie.ac.at
SO MOLECULAR MICROBIOLOGY, (1996 Feb) 19 (3) 495-503.
Journal code: MOM; 8712028. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199610
ED Entered STN: 19961025
Last Updated on STN: 19961025
Entered Medline: 19961016
AB The cell surface of Bacillus stearothermophilus PV72 is covered by a regular surface layer (S-layer) composed of single species of protein, SbsA, with a molecular weight of 130,000. Recently, the sequence of the corresponding gene (sbsA) has been determined. The SbsA coding region including the signal sequence was cloned as a polymerase chain reaction (PCR) product into a low-copy-number vector under the transcriptional control of the lambda pL promoter. Expression of sbsA was shown to be thermally inducible from the resulting vector pBK4 in a strain of Escherichia coli expressing the lambda cI857 from the chromosome. As shown by ultrathin sectioning of whole cells and immunogold labelling using SbsA- specific antibodies, expression of sbsA in E. coli led to accumulation of sheet-like self-assembling products of the protein in the cytoplasm. No SbsA protein was detected either in the periplasm or in the supernatant fractions. Long-term expression of sbsA from pBK4, including in the late stationary phase, did not lead to degradation of SbsA.

=> s s layer protein or s-layer protein

6 FILES SEARCHED...
L30 996 S LAYER PROTEIN OR S-LAYER PROTEIN

=> s l30 and bacteri?

L31 729 L30 AND BACTERI?

=> s l31 and (fusion or recombinant)

L32 148 L31 AND (FUSION OR RECOMBINANT)

=> dup rem l32

PROCESSING COMPLETED FOR L32
L33 66 DUP REM L32 (82 DUPLICATES REMOVED)

=> d bib ab 1-66

L33 ANSWER 1 OF 66 USPATFULL. DUPLICATE 1
AN 2001:47822 USPATFULL
TI Expression and secretion of heterologous polypeptides from caulobacter
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
Nomellini, John F., Richmond, Canada
PA The University of British Columbia, Canada (non-U.S. corporation)
PI US 6210948 B1 20010403
WO 9734000 19970918
AI US 1999-142648 19990330 (9)
WO 1997-CA167 19970310
19990330 PCT 371 date
19990330 PCT 102(e) date
RLI Continuation-in-part of Ser. No. US 1996-614377, filed on 12 Mar 1996, now patented, Pat. No. US 5976864 Continuation-in-part of Ser. No. US 1994-194290, filed on 9 Feb 1994, now patented, Pat. No. US 5500353 Continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Nashed, Nashaat T.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 23
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 1362
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB DNA constructs are provided which code for a chimeric protein in which the C-terminal region corresponds to the extreme C-terminal amino acids of a Caulobacter ***S*** - ***layer*** ***protein*** , fused with a heterologous polypeptide. ***Bacterial*** cells containing the DNA constructs, or which express the DNA constructs and secrete the resulting protein, are provided. Chimeric proteins including the C-terminal amino acids of a Caulobacter ***S*** - ***layer*** ***protein*** are provided, including proteins which include antigenic epitopes of the Infectious Hematopoietic Necrosis Virus.

L33 ANSWER 2 OF 66 USPATFULL
AN 2001:82520 USPATFULL
TI Acid-inducible promoters for gene expression
IN Kullen, Martin J., Raleigh, NC, United States
Klaenhammer, Todd R., Raleigh, NC, United States
PA North Carolina State University, Raleigh, NC, United States (U.S. corporation)
PI US 6242194 B1 20010605
AI US 2000-637968 20000811 (9)

RLI Continuation of Ser. No. US 1999-336861, filed on 21 Jun 1999, now abandoned
DT Utility
EXNAM Primary Examiner: LeGuyader, John L.; Assistant Examiner: Zara, Jane
LREP Myers Bigel Sibley & Sajovec
CLMN Number of Claims: 22
ECL Exemplary Claim: 16
DRWN 8 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 951
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An isolated polynucleotide encoding an acid-inducible, or acid-responsive, promoter element includes the F.sub.1 F.sub.0 -ATPase promoter of Lactobacillus acidophilus DNA that hybridizes thereto and encodes an acid-inducible promoter. ***Recombinant*** molecules comprising the promoter operatively associated with a DNA of interest, along with vectors and host cells containing the same, are also disclosed. Methods of upregulating the transcription of a DNA of interest in a host cell with such promoters are also disclosed.

L33 ANSWER 3 OF 66 USPATFULL
AN 2001:78932 USPATFULL
TI Thermophilic polymerase III holoenzyme
IN McHenry, Charles S., Denver, CO, United States
Seville, Mark, Denver, CO, United States
Cull, Millard G., Denver, CO, United States
PA University Technology Corporation, CO, United States (U.S. corporation)
PI US 6238905 B1 20010529
AI US 1997-928213 19970912 (8)
DT Utility
EXNAM Primary Examiner: Stole, Einar
LREP Medlen & Carroll, LLP
CLMN Number of Claims: 39
ECL Exemplary Claim: 1
DRWN 29 Drawing Figure(s); 18 Drawing Page(s)
LN.CNT 4725
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to gene and amino acid sequences encoding DNA polymerase III holoenzyme subunits and structural genes from thermophilic organisms. In particular, the present invention provides DNA polymerase III holoenzyme subunits of T. thermophilus. The present invention also provides antibodies and other reagents useful to identify DNA polymerase III molecules.

L33 ANSWER 4 OF 66 CAPLUS COPYRIGHT 2001 ACS
AN 2001:247521 CAPLUS
DN 134:291099
TI ***Recombinant*** expression and extracellular secretion of exogenous proteins in coryneform ***bacteria*** by protease cleavage of proprotein-signal peptide ***fusion*** construct
IN Kikuchi, Yoshimi; Date, Masayo; Umezawa, Yukiko; Yokoyama, Keiichi; Matsui, Hiroshi
PA Ajinomoto Co., Inc., Japan
SO PCT Int. Appl., 151 pp.
CODEN: PIXXD2
DT Patent

LA Japanese
FAN.CNT 1
PATENT NO. KIND DATE APPLICATION NO. DATE
PI WO 2001023591 A1 20010405 WO 2000-JP6780 20000929
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
PRAI JP 1999-280098 A 19990930
JP 2000-194043 A 20000628
AB A process for the prodn. of a exogenous secretory protein by using a coryneform ***bacterium*** is disclosed. The method comprises making a coryneform ***bacterium*** to produce an industrially useful exogenous protein (in particular, transglutaminase) and efficiently secreting the product outside the cells (i.e., secretion). A target exogenous protein is produced by using an expression construct wherein the target exogenous protein gene sequence contg. the pro-structure part is ligated to the downstream of a sequence encoding the signal peptide originating in a coryneform ***bacterium*** , transferring this expression type gene construct into the coryneform ***bacterium*** , culturing the thus transformed coryneform ***bacterium*** , and treating the extracellularly released protein with a protease, etc. to cleave and eliminate the pro-part. Use of the signal peptide of ***S*** - ***layer*** ***protein*** (S-protein) such as Corynebacterium ammoniagenes slpA or Corynebacterium glutamicum PS1 and PS2, with a Streptomyces albogriseolus serine protease SAM-P45 and Streptomyces mobaraense proline-specific peptidase svPEP, for the prodn. of Streptoverticillium mobaraense or Streptoverticillium cinnamoneum pro-transglutaminase, is described. Streptomyces mobaraense proline-specific peptidase svPEP, active toward Ala-Ala-Pro-pNA, Ala-Phe-Pro-pNA, and Phe-Arg-Ala-Pro-pNA, and inhibited by phenylmethyl sulfonyl fluoride (PMSF) or aminoethyl benzene sulfonyl fluoride hydrochloride, is specifically used.
RE.CNT 12
RE
(1) Ajinomoto Co Inc; JP 10108675 A 1998 CAPLUS
(2) Duran, R; Biochimie 1998, V80(4), P313 CAPLUS
(3) Kim, I; THE JOURNAL OF BIOLOGICAL CHEMISTRY 1993, V268(17), P12682 CAPLUS
(4) Mercian Corporation; JP 05244947 A 1993 CAPLUS
(5) Mitsubishi Chemical Corporation; JP 09316095 A 1997 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L33 ANSWER 5 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 2
AN 2001151955 EMBASE
TI Expression and testing of Pseudomonas aeruginosa vaccine candidate proteins prepared with the Caulobacter crescentus ***S*** - ***layer*** ***protein*** expression system.
AU Umelo-Njaka E.; Nomellini J.F.; Bingle W.H.; Glasier L.G.M.; Irvin R.T.; Smit J.

CS J. Smit, Department of Microbiology, University of British Columbia, 300-6174 University Boulevard, Vancouver, BC V6T 1Z3, Canada. jsmit@interchange.ubc.ca
SO Vaccine, (8 Jan 2001) 19/11-12 (1406-1415).
Refs: 49
ISSN: 0264-410X CODEN: VACCDE
PUI S 0264-410X(00)00362-5
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
017 Public Health, Social Medicine and Epidemiology
026 Immunology, Serology and Transplantation
036 Health Policy, Economics and Management
037 Drug Literature Index
LA English
SL English
AB A novel ***bacterial*** protein secretion system was used to produce vaccine candidates against Pseudomonas aeruginosa. The surface protein (RsaA) of Caulobacter crescentus was adapted to produce ***recombinant*** vaccine proteins based on the pilus tip epitope ('adhesintope') of P. aeruginosa. Two versions of the adhesintope, with (PCK) or without (PE) the cysteine residues that flank the epitope were investigated, fused to the C-terminus or inserted into full-length RsaA. When expressed in caulobacter the ***fusion*** proteins were secreted as aggregates. Full length RsaA-containing adhesintopes assembled on the cell surface as an S-layer; these were recovered by low pH extraction. Vaccine candidates were evaluated in a mouse challenge model. PCK-containing proteins produced at least 1000-fold higher antibody titers against the adhesintope, compared to the PE version, exceeding titers achievable with any other vaccine preparation method. Immunoglobulin isotyping indicated a balanced IgG1/IgG2 response, though when challenged with P. aeruginosa, the PE- and PCK-containing proteins did not afford mice a significant level of protection. Overall, we describe a new system for vaccine production that shows promise as a fast, economical way to construct, evaluate and produce vaccine proteins. .COPYRGT. 2001 Elsevier Science Ltd.

L33 ANSWER 6 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 3
AN 2001183818 EMBASE
TI Analysis of the structure-function relationship of the ***S*** - ***layer*** ***protein*** SbsC of Bacillus stearothermophilus ATCC 12980 by producing truncated forms.
AU Jarosch M.; Egelseer E.M.; Huber C.; Moll D.; Mattanovich D.; Sleytr U.B.; Sara M.
CS M. Sara, Ctr. for Ultrastructure Res./Ludwig, Boltzmann-Inst. Mol. Nanotec., University of Agricultural Sciences, 1180 Vienna, Austria. sara@edv1.boku.ac.at
SO Microbiology, (2001) 147/5 (1353-1363).
Refs: 31
ISSN: 1350-0872 CODEN: MROBEO
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
LA English
SL English

AB The mature surface layer (***S*** - ***layer***) ***protein*** SbsC of Bacillus stearothermophilus ATCC 12980 comprises amino acids 31-1099 and self-assembles into an oblique lattice type which functions as an adhesion site for a cell-associated high-molecular-mass exoamylase. To elucidate the structure-function relationship of distinct segments of SbsC, three N- and seven C-terminal truncations were produced in a heterologous expression system, isolated, purified and their properties compared with those of the ***recombinant*** mature ***S*** - ***layer*** ***protein*** rSbsC(31-1099). With the various truncated forms it could be demonstrated that the N-terminal part (aa 31-257) is responsible for anchoring the S-layer subunits via a distinct type of secondary cell wall polymer to the rigid cell wall layer, but this positively charged segment is not required for the self-assembly of SbsC, nor for generating the oblique lattice structure. If present, the N-terminal part leads to the formation of in vitro double-layer self-assembly products. Affinity studies further showed that the N-terminal part includes an exoamylase-binding site. Interestingly, the N-terminal part carries two sequences of 6 and 7 aa (AKAALD and KAAYEAA) that were also identified on the amylase-binding protein AbpA of Streptococcus gordonii. In contrast to the self-assembling N-terminal truncation rSbsC(258-1099), two further N-terminal truncations (rSbsC(343-1099), rSbsC(447-1099)) and three C-terminal truncations (rSbsC(31-713), rSbsC(31-844), rSbsC(31-860)) had lost the ability to self-assemble and stayed in the water-soluble state. Studies with the self-assembling C-terminal truncations rSbsC(31-880), rSbsC(31-900) and rSbsC(31-920) revealed that the C-terminal 219 aa can be deleted without interfering with the self-assembly process, while the C-terminal 179 aa are not required for the formation of the oblique lattice structure.

L33 ANSWER 7 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 4
AN 2001039112 EMBASE
TI The ***S*** - ***layer*** ***protein*** of Lactobacillus acidophilus ATCC 4356: Identification and characterisation of domains responsible for S-protein assembly and cell wall binding.
AU Smit E.; Oling F.; Demel R.; Martinez B.; Pouwels P.H.
CS P.H. Pouwels, Dept. Appl. Microbiol./Gene Technol., TNO Nutr./Food Research Institute, Utrechtseweg 48, 3700 AJ Zeist, Netherlands. Pouwels@voeding.tno.nl
SO Journal of Molecular Biology, (12 Jan 2001) 305/2 (245-257).
Refs: 46
ISSN: 0022-2836 CODEN: JMOBAK
CY United Kingdom
DT Journal; Article
FS 029 Clinical Biochemistry
LA English
SL English
AB Lactobacillus acidophilus, like many other ***bacteria*** , harbors a surface layer consisting of a protein (S(A)-protein) of 43 kDa. S(A)-protein could be readily extracted and crystallized in vitro into large crystalline patches on lipid monolayers with a net negative charge but not on lipids with a net neutral charge. Reconstruction of the S-layer from crystals grown on dioleoylphosphatidylserine indicated an oblique lattice with unit cell dimensions (a = 118 A; b = 53 A, and .gamma. = 102.degree.) resembling those determined for the S-layer of Lactobacillus helveticus ATCC 12046. Sequence comparison of S(A)-protein with S-proteins

from L. helveticus, Lactobacillus crispatus and the S-proteins encoded by the silent S-protein genes from L. acidophilus and L. crispatus suggested the presence of two domains, one comprising the N-terminal two-thirds (SAN), and another made up of the C-terminal one-third (SAC) of S(A)-protein. The sequence of the N-terminal domains is variable, while that of the C-terminal domain is highly conserved in the S-proteins of these organisms and contains a tandem repeat. Proteolytic digestion of S(A)-protein showed that SAN was protease-resistant, suggesting a compact structure. SAC was rapidly degraded by proteases and therefore probably has a more accessible structure. DNA sequences encoding SAN or Green Fluorescent Protein fused to SAC (GFP-SAC) were efficiently expressed in Escherichia coli. Purified SAN could crystallize into mono and multi-layered crystals with the same lattice parameters as those found for authentic S(A)-protein. A calculated S(A)-protein minus SAN density-difference map revealed the probable location, in projection, of the SAC domain, which is missing from the truncated SAN peptide. The GFP-SAC ***fusion*** product was shown to bind to the surface of L. acidophilus, L. helveticus and L. crispatus cells from which the S-layer had been removed, but not to non-stripped cells or to Lactobacillus casei.

L33 ANSWER 8 OF 66 MEDLINE DUPLICATE 5
AN 2001302162 MEDLINE
DN 21148517 PubMed ID: 11253870
TI ***Recombinant*** vaccines against infectious hematopoietic necrosis virus: production by the Caulobacter crescentus ***S*** - ***layer*** ***protein*** secretion system and evaluation in laboratory trials.
AU Simon B; Nomellini J; Chiou P; Bingle W; Thornton J; Smit J; Leong J A
CS Department of Microbiology and the Center for Salmon Disease Research, Oregon State University, Corvallis 97331-3804, USA.
SO DISEASES OF AQUATIC ORGANISMS, (2001 Jan 26) 44 (1) 17-27. Journal code: C4V; 8807037. ISSN: 0177-5103.
CY Germany: Germany, Federal Republic of
DT (CLINICAL TRIAL)
Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200105
ED Entered STN: 20010604
Last Updated on STN: 20010604
Entered PubMed: 20010319
Entered Medline: 20010531
AB We report the development of an IHNV vaccine produced by a new protein production system based on the ***bacterium*** Caulobacter crescentus. The subunit vaccines that were tested contain a 184 amino acid segment of the IHNV glycoprotein in different ***fusion*** arrangements with the C. crescentus ***S*** - ***layer*** ***protein*** . Relative percent survival of 26 to 34% was demonstrated in rainbow trout fry for a vaccine that contained the 184 amino acid segment of the IHNV glycoprotein fused to the C-terminal one-quarter of the ***S*** - ***layer*** ***protein*** . Inclusion of the universal mammalian T-cell epitopes developed from the measles ***fusion*** protein or the tetanus toxin protein did not increase the effectiveness of the IHNV-G/S-layer ***recombinant*** protein.

L33 ANSWER 9 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 6
AN 2001140541 EMBASE
TI Development of host-vector systems for lactic acid ***bacteria*** .
AU Sung-Sik Y.; Kim C.
CS Y. Sung-Sik, Department of Biological Resources, College of Liberal Arts and Sciences, Yonsei University, Wonju 220-710, Korea, Republic of. sungsik@dragon.yonsei.ac.kr
SO Korean Journal of Applied Microbiology and Biotechnology, (2001) 29/1 (1-11).
Refs: 51
ISSN: 0257-2389 CODEN: SMHAEH
CY Korea, Republic of
DT Journal; General Review
FS 004 Microbiology
LA Korean
SL English; Korean
AB Lactic acid ***bacteria*** (LAB) are widely used for various food fermentations. With the recent advances in modern biotechnology, a variety of bio-products with the high economic values have been produced using microorganisms. For molecular cloning and expression studies on the gene of interest, E. coli has been widely used mainly because vector systems are fully developed. Most plasmid vectors currently used for E. coli carry antibiotic-resistant markers. As it is generally believed that the antibiotic resistance markers are potentially transferred to other ***bacteria*** , application of the plasmid vectors carrying antibiotic resistance genes as selection markers should be avoided, especially for human consumption. By contrast, as LAB have some desirable traits such that they are GRAS(generally recognized as safe), able to secrete gene products out of cell, and their low protease activities, they are regarded as an ideal organism for the genetic manipulation, including cloning and expression of homologous and heterologous genes. However, the vector systems established for LAB are still insufficient to over-produce gene products stably, limiting the use of these organisms for industrial applications. For a past decade, the two popular plasmid vectors, pAM.beta.1 of Streptococcus faecalis and pGK12, the B. subtilis-E. coli shuttle vector derived from pWV01 of Lactococcus lactis ssp. cremoris Wg2, were most widely used to construct efficient chimeric vectors to be stably maintained in many industrial strains of LAB. Currently, non-antibiotic markers such as nisin resistance(Nis(1)) are explored for selecting ***recombinant*** clone. In addition, a gene encoding ***S*** - ***layer*** ***protein*** , slpA, on ***bacterial*** cell wall was successfully recombined with the proper LAB vectors for excretion of the heterologous gene product from LAB. Many food-grade host vector systems were successfully developed, which allowed stable integration of multiple plasmid copies in the chromosome of LAB. More recently, an integration vector system based on the site-specific integration apparatus of temperate lactococcal ***bacteriophage*** , containing the integrase gene(int) and phage attachment site(attP), was published. In conclusion, when various vector systems, which are maintain stably and expressed strongly in LAB, are developed, lots of such food products as enzymes, pharmaceuticals, bioactive food ingredients for human consumption would be produced at a full scale in LAB.

L33 ANSWER 10 OF 66 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-05880 BIOTECHDS

TI Cleavage of Caulobacter produced ***recombinant*** ***fusion*** proteins useful for producing vaccine peptides; including ***recombinant*** human and animal therapeutic antibiotic and vaccine peptides, enzymes, protein polymers and antibacterial enzymes for foodstuffs
AU Smit J
PA Univ.British-Columbia
LO Vancouver, British Columbia, Canada.
PI WO 2000004170 27 Jan 2000
AI WO 1999-CA637 14 Jul 1999
PRAI CA 1998-2237704 14 Jul 1998
DT Patent
LA English
OS WPI: 2000-182434 [16]
AB Cleaving a ***fusion*** consisting of a Caulobacter ***S*** - ***layer*** ***protein*** (containing the C-terminal secretion signal) and a second component heterologous to Caulobacter, using an acid solution, is claimed. Also claimed are: preparing a DNA construct for expression of the ***fusion*** protein; and producing a ***fusion*** protein using the DNA construct. The method is useful for producing pure proteins including ***recombinant*** human and animal therapeutic antibiotic and vaccine peptides, enzymes, protein polymers and antibacterial enzymes for foodstuffs. The method enables economic production of pure proteins, and it reduces the number of purification steps required following fermentation. The aspartate-proline dipeptide is located between the first and second components or adjacent junction between components. The acid solution has a pH range of 1.5-2.5 or 1.65-2.35. The method is carried out at a temperature of 30-50 deg. Cleaved products are preferably separated from the ***fusion*** protein. Oligonucleotides involved in the isolation of polynucleotides are prepared using conventional solid phase techniques. (33pp)

L33 ANSWER 11 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 7
AN 2001123952 EMBASE
TI Expression of cbsA encoding the collagen-binding S-protein of Lactobacillus crispatus JCM5810 in Lactobacillus casei ATCC 393(T).
AU Martinez B.; Sillanpaa J.; Smit E.; Korhonen T.K.; Pouwels P.H.
CS P.H. Pouwels, Department of Applied Microbiology, TNO Voeding, P.O. Box 360, 3700 AJ Zeist, Netherlands. Pouwels@voeding.tno.nl
SO Journal of Bacteriology, (2000) 182/23 (6857-6861).
Refs: 31
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The cbsA gene encoding the collagen-binding ***S*** - ***layer*** ***protein*** of Lactobacillus crispatus JCM5810 was expressed in L. casei ATCC 393(T). The S-protein was not retained on the surface of the ***recombinant*** ***bacteria*** but was secreted into the medium. By translational ***fusion*** of CbsA to the cell wall sorting signal of the proteinase, PrtP, of L. casei, CbsA was presented at the surface, rendering the transformants able to bind to immobilized collagens.

L33 ANSWER 12 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 8
AN 2000388501 EMBASE
TI Characterization of the collagen-binding ***S*** - ***layer*** ***protein*** CbsA of Lactobacillus crispatus.
AU Sillanpaa J.; Martinez B.; Antikainen J.; Toba T.; Kalkkinen N.; Tankka S.; Lounatmaa K.; Keranen J.; Hook M.; Westerlund-Wikstrom B.; Pouwels P.H.; Korhonen T.K.
CS T.K. Korhonen, Division of General Microbiology, Department of Biosciences, University of Helsinki, P.O. Box 56, FIN 00014 Helsinki, Finland. timo.korhonen@helsinki.fi
SO Journal of Bacteriology, (2000) 182/22 (6440-6450).
Refs: 48
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The cbsA gene of Lactobacillus crispatus strain JCM 5810, encoding a protein that mediates adhesiveness to collagens, was characterized and expressed in Escherichia coli. The cbsA open reading frame encoded a signal sequence of 30 amino acids and a mature polypeptide of 410 amino acids with typical features of a ***bacterial*** ***S*** - ***layer*** ***protein*** . The cbsA gene product was expressed as a His tag ***fusion*** protein, purified by affinity chromatography, and shown to bind solubilized as well as immobilized type I and IV collagens. Three other Lactobacillus S-layer proteins, SIpA, CbsB, and SIpnB, bound collagens only weakly, and sequence comparisons of CbsA with these S-layer proteins were used to select sites in cbsA where deletions and mutations were introduced. In addition, hybrid S-layer proteins that contained the N or the C terminus from CbsA, SIpA, or SIpnB as well as N- and C-terminally truncated peptides from CbsA were constructed by gene ***fusion*** . Analysis of these molecules revealed the major collagen-binding region within the N-terminal 287 residues and a weaker type I collagen-binding region in the C terminus of the CbsA molecule. The mutated or hybrid CbsA molecules and peptides that failed to polymerize into a periodic S-layer did not bind collagens, suggesting that the crystal structure with a regular array is optimal for expression of collagen binding by CbsA. Strain JCM 5810 was found to contain another S-layer gene termed cbsB that was 44% identical in sequence to cbsA. RNA analysis showed that cbsA, but not cbsB, was transcribed under laboratory conditions. ***S*** - ***layer*** - ***protein*** -expressing cells of strain JCM 5810 adhered to collagen-containing regions in the chicken colon, suggesting that CbsA-mediated collagen binding represents a true tissue adherence property of L. crispatus.

L33 ANSWER 13 OF 66 MEDLINE DUPLICATE 9
AN 2000270166 MEDLINE
DN 20270166 PubMed ID: 10809716
TI Secretion of the Caulobacter crescentus ***S*** - ***layer*** ***protein*** : further localization of the C-terminal secretion signal and its use for secretion of ***recombinant*** proteins.
AU Bingle W H; Nomellini J F; Smit J
CS Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada.

SO JOURNAL OF BACTERIOLOGY, (2000 Jun) 182 (11) 3298-301.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200006
ED Entered STN: 20000616
Last Updated on STN: 20000616
Entered Medline: 20000605
AB The secretion signal of the Caulobacter crescentus ***S*** - ***layer*** ***protein*** (RsaA) was localized to the C-terminal 82 amino acids of the molecule. Protein yield studies showed that 336 or 242 C-terminal residues of RsaA mediated secretion of >50 mg of a cellulase passenger protein per liter to the culture fluids.

L33 ANSWER 14 OF 66 CAPLUS COPYRIGHT 2001 ACS
AN 2000:476644 CAPLUS
DN 133:345267
TI Gene cloning and expression and secretion of Listeria monocytogenes ***bacteriophage*** -lytic enzymes in Lactococcus lactis
AU Gaeng, Susanne; Scherer, Siegfried; Neve, Horst; Loessner, Martin J.
CS Institut fur Mikrobiologie, FML Weihenstephan, Institut fur Mikrobiologie, FML Weihenstephan, Technische Universitat Munchen, Freising, D-85350, Germany
SO Appl. Environ. Microbiol. (2000), 66(7), 2951-2958
CODEN: AEMIDF; ISSN: 0099-2240
PB American Society for Microbiology
DT Journal
LA English
AB ***Bacteriophage*** lysins (Ply), or endolysins, are phage-encoded cell wall lytic enzymes which are synthesized late during virus multiplication and mediate the release of progeny virions. ***Bacteriophages*** of the pathogen Listeria monocytogenes encode endolysin enzymes which specifically hydrolyze the crosslinking peptide bridges in Listeria peptidoglycan. Ply118 is a 30.8-kDa L-alanyl-D-glutamate peptidase and Ply511 (36.5 kDa) acts as N-acetylmuramoyl-L-alanine amidase. In order to establish dairy starter cultures with biopreservation properties against L. monocytogenes contaminations, we have introduced ply118 and ply511 into Lactococcus lactis MG1363 by using a pTRKH2 backbone. The genes were expressed under control of the lactococcal promoter P32, which proved superior to other promoters (P21 and P59) tested in this study. High levels of active enzymes were produced and accumulated in the cytoplasmic cell fractions but were not released from the cells at significant levels. Therefore, ply511 was genetically fused with the SPslpA nucleotide sequence encoding the Lactobacillus brevis ***S*** - ***layer*** ***protein*** signal peptide. Expression of SPslpA-ply511 from pSL-PL511 resulted in secretion of functional Ply511 enzyme from L. lactis cells. One clone expressed an unusually strong lytic activity, which was found to be due to a 115-bp deletion that occurred within the 3'-end coding sequence of SPslpA-ply511, which caused a frameshift mutation and generated a stop codon. Surprisingly, the resulting carboxy-terminal deletion of 80 amino acids in the truncated Ply511.DELTA.(S262-K341) mutant polypeptide strongly increased its lytic activity. Proteolytic processing of the

secretion competent SPSlpA-Ply511 propeptide following membrane translocation had no influence on enzyme activity. Immunoblotting expts. using both cytoplasmic and supernatant fractions indicated that the enzyme was quant. exported from the cells and secreted into the surrounding medium, where it caused rapid lysis of L. monocytogenes cells. Moreover, transformation of pSL-PL511.DELTA.C into L. lactis Bu2-129, a lactose-utilizing strain that can be employed for fermn. of milk, also resulted in secretion of functional enzyme and showed that the vector is compatible with the native lactococcal plasmids.

RE.CNT 47

RE

(3) Cardinal, M; Food Biotechnol 1997, V11, P129 CAPLUS

(4) Cintas, L; Appl Environ Microbiol 1995, V61, P2643 CAPLUS

(5) Dickely, F; Mol Microbiol 1995, V15, P839 CAPLUS

(7) Dower, W; Nucleic Acids Res 1988, V16, P6127 CAPLUS

(9) Foegeding, P; Appl Environ Microbiol 1992, V58, P884 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L33 ANSWER 15 OF 66 BIOSIS COPYRIGHT 2001 BIOSIS

AN 2000:439659 BIOSIS

DN PREV200000439659

TI ISBst12, a novel type of insertion-sequence element causing loss of S-layer-gene expression in Bacillus stearothermophilus ATCC 12980.

AU Egelseer, Eva M. (1); Idris, Rughia; Jarosch, Marina; Danhorn, Thomas; Sleytr, Uwe B.; Sara, Margit

CS (1) Zentrum fuer Ultrastrukturforschung und Ludwig Bolzmann, Institut fuer Molekulare Nanotechnologie, Universitaet fuer Bodenkultur, A-1180, Vienna Austria

SO Microbiology (Reading), (September, 2000) Vol. 146, No. 9, pp. 2175-2183. print.
ISSN: 1350-0872.

DT Article

LA English

SL English

AB The cell surface of the surface layer (S-layer)-carrying strain of Bacillus stearothermophilus ATCC 12980 is completely covered with an oblique lattice composed of the ***S*** - ***layer*** ***protein*** SbsC. In the S-layer-deficient strain, the S-layer gene sbsC was still present but was interrupted by a novel type of insertion sequence (IS) element designated ISBst12. The insertion site was found to be located within the coding region of the sbsC gene, 199 bp downstream from the translation start of SbsC. ISBst12 is 1612 bp long, bounded by 16 bp imperfect inverted repeats and flanked by a directly repeated 8 bp target sequence. ISBst12 contains an ORF of 1446 bp and is predicted to encode a putative transposase of 482 aa with a calculated theoretical molecular mass of 55562 Da and an isoelectric point of 9.13. The putative transposase does not exhibit a typical DDE motif but displays a His-Arg-Tyr triad characteristic of the active site of integrases from the ***bacteriophage*** lambda Int family. Furthermore, two overlapping leucine-zipper motifs were identified at the N-terminal part of the putative transposase. As revealed by Southern blotting, ISBst12 was present in multiple copies in the Slayer-deficient strain as well as in the S-layer-carrying strain. Northern blotting indicated that S-layer gene expression is already inhibited at the transcriptional level, since no sbsC-specific transcript could be identified in the S-layer-deficient

strain. By using PCR, ISBst12 was also detected in B. stearothermophilus PV72/p6, in its oxygen-induced strain variant PV72/p2 and in the S-layer-deficient strain PV72/T5.

L33 ANSWER 16 OF 66 MEDLINE DUPLICATE 10
AN 2000170659 MEDLINE
DN 20170659 PubMed ID: 10708365
TI S-layer gene sbsC of Bacillus stearothermophilus ATCC 12980: molecular characterization and heterologous expression in Escherichia coli.
AU Jarosch M; Egelseer E M; Mattanovich D; Sleytr U B; Sara M
CS Zentrum fur Ultrastrukturforschung und Ludwig Boltzmann-Institut fur Molekulare Nanotechnologie, Universitat fur Bodenkultur, Vienna, Austria.
SO MICROBIOLOGY, (2000 Feb) 146 (Pt 2) 273-81.
Journal code: BXW; 9430468. ISSN: 1350-0872.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AF055578
EM 200005
ED Entered STN: 20000512
Last Updated on STN: 20000512
Entered Medline: 20000501
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered with an oblique S-layer lattice. To investigate sequence identities and a common structure-function relationship in S-layer proteins of different B. stearothermophilus wild-type strains, the nucleotide sequence encoding the ***S*** - ***layer*** ***protein*** SbsC of B. stearothermophilus ATCC 12980 was determined by PCR techniques. The entire sbsC sequence showed an ORF of 3297 bp predicted to encode a protein of 1099 aa with a theoretical molecular mass of 115409 Da and an isoelectric point of 5.73. Primer extension analysis suggested the existence of two promoter regions. Amino acid sequence comparison between SbsC and SbsA, a previously characterized ***S*** - ***layer*** ***protein*** of B. stearothermophilus PV72/p6 which assembles into a hexagonally ordered lattice, revealed an identical secretion signal peptide, 85% identity for the N-terminal regions (aa 31-270) which do not carry any S-layer homologous motifs, but only 21% identity for the rest of the sequences. Affinity studies demonstrated that the N-terminal part of SbsC is necessary for recognition of a secondary cell wall polymer. This was in accordance with results obtained in a previous study for SbsA, thus confirming a common functional principle for the N-terminal parts of both S-layer proteins. The sbsC coding region cloned into the pET3a vector without its own upstream region, the signal sequence and the 3' transcriptional terminator led to stable expression in Escherichia coli.

L33 ANSWER 17 OF 66 BIOSIS COPYRIGHT 2001 BIOSIS
AN 2000:418709 BIOSIS
DN PREV200000418709
TI The transposable element IS4712 prevents S-layer gene (sbsA) expression in Bacillus stearothermophilus and also affects the synthesis of altered surface layer proteins.
AU Scholz, Holger (1); Hummel, Susanne; Witte, Angela; Lubitz, Werner; Kuen, Beatrix

CS (1) Institute of Animal Hygiene and Public Veterinary Health, An den Tierkliniken 43, 04103, Leipzig Germany
SO Archives of Microbiology, (July August, 2000) Vol. 174, No. 1-2, pp. 97-103. print.
ISSN: 0302-8933.
DT Article
LA English
SL English
AB Cell surface (***S***)- ***layer*** ***protein*** synthesis in Bacillus stearothermophilus PV72/p6 is blocked when cells are grown at elevated temperature. From a culture exhibiting the S-layer-negative phenotype, the S-layer deficient mutant T5 (SbsA-) was isolated. Genetic analysis of the S-layer-encoding gene (sbsA) of mutant T5 revealed an insertion element (IS4712) integrated into the upstream regulatory region of the S-layer gene, thereby blocking sbsA transcription. The insertion element consists of 1371 base pairs which are flanked by two perfect inverted terminal repeats. Sequence similarity to other transposases of the IS4 family was detected. DNA-DNA hybridizations demonstrated that multiple homologues of IS4712 were also present within the genomes of several other thermophilic bacillus isolates. Attempts to isolate SbsA+ revertants failed. Instead, cells with altered surface proteins were detected. The synthesis of the altered S-layer proteins was correlated with the presence of IS4712 along with the occurrence of deletions in the sbsA coding region. Furthermore imprecise excision of IS4712 was detected. This work demonstrated that B. stearothermophilus is able to express at least four different S-layer proteins and that blocking of sbsA transcription by the insertion element IS4712 is associated with the expression of altered surface proteins.

L33 ANSWER 18 OF 66 USPATFULL DUPLICATE 11
AN 1999:137013 USPATFULL
TI Expression and secretion of heterologous polypeptides from caulobacter
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
Nomellini, John F., Richmond, Canada
PA The University of British Columbia, Canada (non-U.S. corporation)
PI US 5976864 19991102
AI US 1996-614377 19960312 (8)
RLI Continuation-in-part of Ser. No. US 1994-194290, filed on 9 Feb 1994, now patented, Pat. No. US 5500353 which is a continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Nashed, Nashaat T.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 14
ECL Exemplary Claim: 2
DRWN 14 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 1609
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB DNA constructs are provided which code for at least the extreme C-terminal amino acids of the rsaA protein of Caulobacter crescentus fused with heterologous polypeptides. Baterial cells containing, or which express the DNA constructs and secrete the resulting protein are also provided. Chimeric proteins including the C-terminal amino acids of the rsaA protein are provided, including chimeric proteins comprising

antigenic epitopes of the Infectious Hematopoietic Necrosis Virus.

L33 ANSWER 19 OF 66 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 12
AN 1999:659414 CAPLUS
DN 131:282413
TI Lactobacillus epithelial cell-binding ***S*** - ***layer*** ***protein*** and slpA gene and ***recombinant*** Lactobacillus probiotics and vaccines
IN Korhonen, Timo; Palva, Airi; Palva, Ilkka; Hynonen, Ulla; Westerlund-Wikstrom, Benita
PA Finland
SO PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9951631	A1	19991014	WO 1999-FI290	19990406
FI 9800782	A	19991004	FI 1998-782	19980403
AU 9933343	A1	19991025	AU 1999-33343	19990406

PI WO 9951631
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI FI 1998-782 19980403
WO 1999-FI290 19990406

AB This invention relates to the L. brevis slpA gene and the encoded ***S*** - ***layer*** ***protein*** which is involved in ***bacterial*** adhesion to mammalian epithelium. It is possible to modify or improve the binding capacity of various prokaryotic or eucaryotic cells to human and/or animal epithelial cell types, such as intestinal, urogenital and/or endothelial cells, by using lactobacillar surface structures of this invention. In particular, it is possible with the nucleotide sequences of this invention to improve the binding properties of a host cell having probiotic effects to human and/or animal epithelial cell types. Thus, chimeric slpA-fliC genes encoding various fragments of the SlpA protein fused to the FliC flagellar protein were expressed in Escherichia coli. The binding of the ***recombinant*** E. coli to intestinal and urogenital cells was detd. Adhesion activity was localized to the N-terminus of the SlpA protein. The shortest subfragment of SlpA which bound to the epithelial cells was amino acids 96-200.

RE.CNT 5
RE
(1) Bahl, H; FEMS Microbiology Reviews 1997, V20, P47 CAPLUS
(2) Kirsi, S; Gene 1997, V186(2), P255 CAPLUS
(3) Schneitz, C; Journal of Applied Bacteriology 1993, V74, P290 MEDLINE
(4) Viagen, O; WO 9400581 A1 1994 CAPLUS
(5) Vidgren, G; Journal of Bacteriology 1992, V174(22), P7419 CAPLUS

L33 ANSWER 20 OF 66 USPATFULL
AN 1999:24489 USPATFULL
TI Expression of surface layer proteins
IN Deblaere, Rolf Y., Waarschoot, Belgium
Desomer, Jan, Drongen, Belgium
Dhaese, Patrick, Drongen, Belgium
PA Solvay (Societe Anonyme), Brussels, Belgium (non-U.S. corporation)
PI US 5874267 19990223
WO 9519371 19950720
AI US 1996-682517 19960917 (8)
WO 1995-EP147 19950113
19960917 PCT 371 date
19960917 PCT 102(e) date
PRAI GB 1994-650 19940114
DT Utility
EXNAM Primary Examiner: Hutzell, Paula K.; Assistant Examiner: Bakalyar, Heather A.
LREP McDermott, Will & Emery
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 41 Drawing Figure(s); 37 Drawing Page(s)
LN.CNT 2742
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A host cell which is provided with a S-layer comprising a ***fusion*** polypeptide consisting essentially of:

(a) at least sufficient of a ***S*** - ***layer*** ***protein*** for a S-layer composed thereof to assemble, and

(b) a heterologous polypeptide which is fused to either the carboxy terminus of (a) or the amino terminus of (a) and which is thereby presented on the outer surface of the said cell; can be used as a vaccine, for screening for proteins and antigens and as a support for immobilizing an enzyme, peptide or antigen. A process of transforming B. Sphaericus cells comprising electroporation is also provided.

L33 ANSWER 21 OF 66 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1999-04719 BIOTECHDS
TI Producing S-layer proteins in Gram-negative ***bacteria*** or eukaryotes;
for use as ***recombinant*** vaccine
AU Lubitz W
PA Lubitz W
LO Vienna, Austria.
PI DE 19732829 4 Feb 1999
AI DE 1997-1032829 30 Jul 1997
PRAI DE 1997-1032829 30 Jul 1997
DT Patent
LA German
OS WPI: 1999-122189 [11]
AB A means of producing ***S*** - ***layer*** ***protein*** (I) is claimed. It involves transforming a Gram-negative prokaryotic cell with a nucleic acid that encodes (I) linked to a signal peptide that encodes a protein which causes integration of (I) into the external or cytoplasmic

membrane, or secretion of (I) into the periplasmic space or extracellular medium. The ***bacterium*** is then cultured, and (I) recovered from the membrane, periplasmic space, or medium. Alternatively a eukaryotic cell can be used as the host, in which case the signal peptide promotes integration of (I) into the cytoplasmic membrane, or an organelle, or induces secretion of (I) into the extracellular medium. Also claimed is a nucleic acid (II) that encodes (I) and the signal peptide, optionally including heterologous peptide inserts. The claims also cover a vector containing (II), and Gram-positive prokaryotic or eukaryotic cells transformed by that vector (e.g. plasmid pMAL-A used to transform Escherichia coli DH5-alpha. (I) are useful as vaccines, reactors, and universal carrier molecules. (33pp)

L33 ANSWER 22 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 13
AN 1999298549 EMBASE
TI Cell surface-exposed tetanus toxin fragment C produced by ***recombinant*** Bacillus anthracis protects against tetanus toxin.
AU Mesnage S.; Weber-Levy M.; Haustant M.; Mock M.; Fouet A.
CS S. Mesnage, Toxines et Pathogenie Bacteriennes, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris Cedex 15, France. smesnage@pasteur.fr
SO Infection and Immunity, (1999) 67/9 (4847-4850).
Refs: 30
ISSN: 0019-9567 CODEN: INFIBR
CY United States
DT Journal; Article
FS 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB Bacillus anthracis, the causal agent of anthrax, synthesizes two surface layer (S-layer) proteins, EA1 and Sap, which account for 5 to 10% of total protein and are expressed in vivo. A ***recombinant*** B. anthracis strain was constructed by integrating into the chromosome a translational ***fusion*** harboring the DNA fragments encoding the cell wall-targeting domain of the ***S*** - ***layer*** ***protein*** EA1 and tetanus toxin fragment C (ToxC). This construct was expressed under the control of the promoter of the S-layer component gene. The hybrid protein was stably expressed on the cell surface of the ***bacterium*** . Mice were immunized with bacilli of the corresponding strain, and the hybrid protein elicited a humoral response to ToxC. This immune response was sufficient to protect mice against tetanus toxin challenge. Thus, the strategy developed in this study may make it possible to generate multivalent live veterinary vaccines, using the ***S*** - ***layer*** ***protein*** genes as a cell surface display system.

L33 ANSWER 23 OF 66 MEDLINE
AN 1999255555 MEDLINE
DN 99255555 PubMed ID: 10322032
TI Three surface layer homology domains at the N terminus of the Clostridium cellulovorans major cellulosomal subunit EngE.
AU Tamaru Y; Doi R H
CS Section of Molecular and Cellular Biology, University of California, Davis, California 95616, USA.
SO JOURNAL OF BACTERIOLOGY, (1999 May) 181 (10) 3270-6.

Journal code: HH3; 2985120R. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AF105331
EM 199906
ED Entered STN: 19990628
Last Updated on STN: 19990628
Entered Medline: 19990617
AB The gene engE, coding for endoglucanase E, one of the three major subunits of the Clostridium cellulovorans cellulosome, has been isolated and sequenced. engE is comprised of an open reading frame (ORF) of 3,090 bp and encodes a protein of 1,030 amino acids with a molecular weight of 111,796. The amino acid sequence derived from engE revealed a structure consisting of catalytic and noncatalytic domains. The N-terminal-half region of EngE consisted of a signal peptide of 31 amino acid residues and three repeated surface layer homology (SLH) domains, which were highly conserved and homologous to an ***S*** - ***layer*** ***protein*** from the gram-negative ***bacterium*** Caulobacter crescentus. The C-terminal-half region, which is necessary for the enzymatic function of EngE and for binding of EngE to the scaffolding protein CbpA, consisted of a catalytic domain homologous to that of family 5 of the glycosyl hydrolases, a domain of unknown function, and a duplicated sequence (DS or dockerin) at its C terminus. engE is located downstream of an ORF, ORF1, that is homologous to the Bacillus subtilis phosphomethylpyrimidine kinase (pmk) gene. The unique presence of three SLH domains and a DS suggests that EngE is capable of binding both to CbpA to form a CbpA-EngE cellulosome complex and to the surface layer of C. cellulovorans.

L33 ANSWER 24 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 14
AN 1999138054 EMBASE
TI Cloning and characterization of two bistructural S-layer-RTX proteins from Campylobacter rectus.
AU Braun M.; Kuhnert P.; Nicolet J.; Burnens A.P.; Frey J.
CS J. Frey, Inst. for Veterinary Bacteriology, Langgasstrasse 122, CH-3012 Berne, Switzerland. jfrey@vbi.unibe.ch
SO Journal of Bacteriology, (1999) 181/8 (2501-2506).
Refs: 46
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Campylobacter rectus is an important periodontal pathogen in humans. A surface-layer (***S*** - ***layer***) ***protein*** and a cytotoxic activity have been characterized and are thought to be its major virulence factors. The cytotoxic activity was suggested to be due to a pore-forming protein toxin belonging to the RTX (repeats in the structural toxins) family. In the present work, two closely related genes, csxA and csxB (for C. rectus S- layer and RTX protein) were cloned from C. rectus and characterized. The Csx proteins appear to be bifunctional and possess two structurally different domains. The N-terminal part shows similarity with ***S*** - ***layer*** ***protein*** , especially SapA and

SapB of C. fetus and Crs of C. rectus. The C-terminal part comprising most of CsxA and CsxB is a domain with 48 and 59 glycine- rich canonical nonapeptide repeats, respectively, arranged in three blocks. Purified ***recombinant*** Csx peptides bind Ca2+. These are characteristics traits of RTX toxin proteins. The S-layer and RTX domains of Csx are separated by a proline-rich stretch of 48 amino acids. All C. rectus isolates studied contained copies of either the csxA or csxB gene or both; csx genes were absent from all other Campylobacter and Helicobacter species examined. Serum of a patient with acute gingivitis showed a strong reaction to ***recombinant*** Csx protein on immunoblots.

L33 ANSWER 25 OF 66 MEDLINE DUPLICATE 15
AN 1999157595 MEDLINE
DN 99157595 PubMed ID: 10048035
TI Production and cell surface anchoring of functional fusions between the SLH motifs of the Bacillus anthracis S-layer proteins and the Bacillus subtilis levansucrase.
AU Mesnage S; Tosi-Couture E; Fouet A
CS Toxines et Pathogenie Bacteriennes (URA 1858, CNRS), Paris, France.. smesnage@pasteur.fr
SO MOLECULAR MICROBIOLOGY, (1999 Feb) 31 (3) 927-36.
Journal code: MOM; 8712028. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199905
ED Entered STN: 19990517
Last Updated on STN: 19990517
Entered Medline: 19990506
AB Many surface proteins of Gram-positive ***bacteria*** contain motifs, about 50 amino acids long, called S-layer homology (SLH) motifs. Bacillus anthracis, the causal agent of anthrax, synthesizes two S-layer proteins, each with three SLH motifs towards the amino-terminus. We used biochemical and genetic approaches to investigate the involvement of these motifs in cell surface anchoring. Proteinase K digestion produced polypeptides lacking these motifs, and stable three-motif polypeptides were produced in Escherichia coli that were able to bind the B. anthracis cell walls in vitro, demonstrating that the three SLH motifs were organized into a cell surface anchoring domain. We also determined the function of these SLH domains by constructing chimeric genes encoding the SLH domains fused to the normally secreted levansucrase of Bacillus subtilis. Cell fractionation and electron microscopy studies showed that each three-motif domain was sufficient for the efficient anchoring of levansucrase onto the cell surface. Proteins consisting of truncated SLH domains fused to levansucrase were unstable and associated poorly with the cell surface. Surface-exposed levansucrase retained its enzymatic and antigenic properties.

L33 ANSWER 26 OF 66 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1999-11466 BIOTECHDS
TI Extended ***recombinant*** ***bacterial*** ghost system; ghost cell production and foreign gene and antigen expression for use as a ***recombinant*** combination vaccine (conference paper)
AU Lubitz W; Witte A; Eko F O; Kamal M; Jechlinger W; Brand E; Marchart J;

Haidinger W; Huter V; Felnerova D; Stralis-Alves N; Lechleitner S; Melzer H; Szostak M P; Resch S; Nader H; Kuen B; Mayr B; Mayrhofer P; Geretschlager R; Haslberger A; Hensel A

CS Univ.Vienna-Inst.Microbiol.Genet.; EVAX-Technol.; Univ.Leipzig-Inst.Anim.Hyg.Vet.Public-Health

LO Institute of Microbiology and Genetics, University of Vienna, Dr. Bohrgasse 9, A-1030 Vienna, Austria.
Email: oldfox@gem.univie.ac.at

SO J.Biotechnol.; (1999) 73, 2-3, 261-73
CODEN: JBITD4 ISSN: 0168-1656
New Approaches in Vaccine Development 1997, Australian Society of Biotechnology, Vienna, Austria, 1997.

DT Journal

LA English

AB Controlled expression of cloned PhiX174 gene E in Gram-negative ***bacteria*** results in lysis of the ***bacteria*** by formation of an E-specific transmembrane tunnel structure built through the cell envelope complex. These ***bacterial*** ghosts from a variety of ***bacteria*** were used as non-living candidate vaccines. In a ***recombinant*** ghost system, the desired foreign proteins are attached to the inside of the inner membrane as fusions with specific anchor sequences. Because the ghosts have a sealed periplasmic and the proteins can be exported into this space the capacity of the ghost or ***recombinant*** ghost systems can be vastly extended, therefore making them capable carriers of foreign antigens. The ***recombinant*** ghosts can also express ***S*** - ***layer*** ***protein*** (shell-like structure), which can carry foreign gene epitopes, which further extends the possibilities of ghost carriers. The ghost also have inherent adjuvant properties, so they can be used as adjuvants in combination with subunit vaccines. There is no limitations on the size of foreign antigens which can be inserted into the ghosts and so they may be used as adjuvant free combination vaccines. (32 ref)

L33 ANSWER 27 OF 66 MEDLINE

AN 1999204063 MEDLINE

DN 99204063 PubMed ID: 10188248

TI Self-assembly product formation of the Bacillus stearothermophilus PV72/p6 ***S*** - ***layer*** ***protein*** SbsA in the course of autolysis of Bacillus subtilis.

AU Howorka S; Sara M; Lubitz W; Kuen B

CS Institut fur Mikrobiologie and Genetik, Universitat Wien, Vienna, Austria.

SO FEMS MICROBIOLOGY LETTERS, (1999 Mar 15) 172 (2) 187-96.
Journal code: FML; 7705721. ISSN: 0378-1097.

CY Netherlands

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199904

ED Entered STN: 19990511
Last Updated on STN: 19990511
Entered Medline: 19990429

AB In order to achieve high level expression and to study the release of a protein capable of self-assembly, the gene encoding the crystalline cell surface (***S*** - ***layer***) ***protein*** SbsA of Bacillus stearothermophilus PV72/p6, including its signal sequence, was cloned and

expressed in Bacillus subtilis. To obtain high level expression, a tightly regulated, xylose-inducible, stably replicating multicopy-plasmid vector was constructed. After induction of expression, the ***S*** - ***layer*** ***protein*** made up about 15% of the total cellular protein content, which was comparable to the SbsA content of B. stearothermophilus PV72/p6 cells. During all growth stages, SbsA was poorly secreted to the ambient cellular environment by B. subtilis. Extraction of whole cells with guanidine hydrochloride showed that in late stationary growth phase cells 65% of the synthesised SbsA was retained in the peptidoglycan-containing layer, indicating that the rigid cell wall layer was a barrier for efficient SbsA secretion. Electron microscopic investigation revealed that SbsA release from the peptidoglycan-containing layer started in the late stationary growth phase at distinct sites at the cell surface leading to the formation of extracellular self-assembly products which did not adhere to the cell wall surface. In addition, intracellular sheet-like SbsA self-assembly products which followed the curvature of the cell became visible in partly lysed cells. Intracellularly formed self-assembly products remained intact even after complete lysis of the rigid cell envelope layer.

L33 ANSWER 28 OF 66 CAPLUS COPYRIGHT 2001 ACS
AN 1999:144110 CAPLUS
TI Adapting the ***bacterium*** Caulobacter crescentus to produce ***recombinant*** proteins using the ***S*** - ***layer*** ***protein*** secretion apparatus
AU Smit, John
CS University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SO Book of Abstracts, 217th ACS National Meeting, Anaheim, Calif., March 21-25 (1999), BIOT-096 Publisher: American Chemical Society, Washington, D. C.
CODEN: 67GHA6
DT Conference; Meeting Abstract
LA English
AB We have developed the ***bacterium*** Caulobacter crescentus for ***recombinant*** protein prodn., at levels readily suitable for research and potentially scalable for com. levels. C. crescentus strains are non-pathogenic ***bacteria*** . They secrete large amts. of a large hydrophilic protein (the Surface [***S***]- ***layer*** ***protein***) which assembles into a geometric pattern on the cell surface. The protein is secreted by a "Type I" mechanism, which is noted for the ability to secrete a wide variety of proteins types (including large and hydrophilic proteins), at levels needed to cover the entire surface (10-12% of total protein). We have adapted the secretion system to produce ***recombinant*** proteins for a variety of needs. Other applications, including presentation of peptides on the surface of the ***bacterium*** (as part of the surface crystal), or applying the protein crystn. capabilities for nanotechnol. are also under development.

L33 ANSWER 29 OF 66 MEDLINE DUPLICATE 16
AN 1999177548 MEDLINE
DN 99177548 PubMed ID: 10077822
TI The expression signals of the Lactobacillus brevis slpA gene direct efficient heterologous protein production in lactic acid ***bacteria***

AU Kahala M; Palva A

CS Agricultural Research Centre of Finland, Food Research Institute, Finland.
SO APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (1999 Jan) 51 (1) 71-8.
Journal code: AMC; 8406612. ISSN: 0175-7598.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199904
ED Entered STN: 19990420
Last Updated on STN: 20000303
Entered Medline: 19990402
AB A cassette based on the expression signals of the Lactobacillus brevis surface (***S***)- ***layer*** ***protein*** gene (slpA) was constructed. The low-copy-number vector pKTH2095, derived from pGK12, was used as the cloning vector. The efficiency of slpA promoters in intracellular protein production was studied using three reporter genes, beta-glucuronidase (gusA), luciferase (luc) and aminopeptidase N (pepN) in three different lactic acid ***bacteria*** hosts: Lactococcus lactis, Lactobacillus plantarum and Lactobacillus gasseri. The S-layer promoters were recognized in each strain and especially L. lactis and Lb. plantarum exhibited high levels of transcripts. The production kinetics of reporter proteins was studied as a function of growth. The GusA, Luc and PepN activities varied considerably among the lactic acid ***bacterial*** strains studied. The highest levels of beta-glucuronidase and luciferase activity were obtained in L. lactis. The level of GusA obtained in L. lactis corresponded to over 15% of the total cellular proteins. The highest level of aminopeptidase N activity was achieved in Lb. plantarum where PepN corresponded up to 28% of the total cellular proteins at the late exponential phase of growth. This level of PepN activity is 30-fold higher than that in Lb. helveticus, which is the species from which the pepN gene originates.

L33 ANSWER 30 OF 66 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1999:161526 BIOSIS
DN PREV199900161526
TI Adapting the ***bacterium*** Caulobacter crescentus to produce ***recombinant*** proteins using the ***S*** - ***layer*** ***protein*** secretion apparatus.
AU Smith, John (1)
CS (1) Univ. British Columbia, Vancouver, BC V6T 1Z3 Canada
SO Abstracts of Papers American Chemical Society, (1999) Vol. 217, No. 1-2, pp. BIOT 096.
Meeting Info.: 217th National Meeting of the American Chemical Society Anaheim, California, USA March 21-25, 1999 American Chemical Society . ISSN: 0065-7727.
DT Conference
LA English

L33 ANSWER 31 OF 66 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1999-14343 BIOTECHDS
TI Adapting the ***bacterium*** Caulobacter crescentus to produce ***recombinant*** proteins using the ***S*** - ***layer*** ***protein*** secretion apparatus;
recombinant protein production (conference abstract)
AU Smit J

CS Univ.British-Columbia
LO University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada.
SO Abstr.Pap.Am.Chem.Soc.; (1999) 217 Meet., Pt.1, BIOT096
CODEN: ACSRAL ISSN: 0065-7727
217th ACS National Meeting, American Chemical Society, Anaheim, CA, USA, 21-25 March, 1999.
DT Journal
LA English
AB Caulobacter crescentus was developed for ***recombinant*** protein production at levels readily suitable for research and potentially scalable for commercial levels. C. crescentus strains are non-pathogenic ***bacteria*** . They secrete large amounts of large hydrophilic protein (the surface (***S***)- ***layer*** ***protein***) which assembles into a geometric pattern on the cell surface. The protein is secreted by a type-I mechanism, which is noted for the ability to secrete a wide variety of protein type (including large and hydrophilic proteins), at levels needed to cover the entire surface (10-12% of total protein). The secretion system was adapted to produce ***recombinant*** proteins for a variety of needs. Other applications, including presentation of peptides on the surface of the ***bacterium*** (as part of the surface crystal), or applying to protein crystallization capabilities for nanotechnology are also under development. (0 ref)

L33 ANSWER 32 OF 66 USPATFULL DUPLICATE 17
AN 1998:85822 USPATFULL
TI Gene and protein applicable to the preparation of vaccines for rickettsia prowazekii and rickettsia typhi and the detection of both
IN Carl, Mitchell, San Diego, CA, United States
Dobson, Michael E., Rockville, MD, United States
Ching, Wei-Mei, Bethesda, MD, United States
Dasch, Gregory A., Wheaton, MD, United States
PA The United States of America as represented by the Secretary of the Navy, Washington, DC, United States (U.S. government)
PI US 5783441 19980721
AI US 1993-169927 19931220 (8)
RLI Continuation-in-part of Ser. No. US 1991-742128, filed on 9 Aug 1991, now abandoned
DT Utility
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Shaver, Jennifer
LREP Spevack, A. David, Garvert, William C.
CLMN Number of Claims: 2
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 928
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB All or part of the DNA sequence of the gene which encodes the ***S*** - ***layer*** ***protein*** of R. prowazekii as illustrated in Sequence ID No. 1 as well as a truncated identical piece of this gene in R. typhi as well as the 5' and 3' noncoding regions can be used for vaccination against typhus and spotted fever rickettsial infection or to diagnose the diseases caused by these ***bacteria*** . The invention is also accomplished by the deduced amino acid sequence of the ***S*** - ***layer*** ***protein*** of R. prowazekii derived from the DNA

sequence of the encoding gene. Further, the invention includes the peptide or protein products based on all or parts of this gene.

L33 ANSWER 33 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 18
AN 1998206517 EMBASE
TI The Caulobacter crescentus paracrystalline ***S*** - ***layer*** ***protein*** is secreted by an ABC transporter (Type I) secretion apparatus.
AU Awram P.; Smit J.
CS J. Smit, Dept. of Microbiology and Immunology, University of British Columbia, 300-6174 University Blvd., Vancouver, BC V6T 1Z3, Canada. jsmit@unixg.ubc.ca
SO Journal of Bacteriology, (1998) 180/12 (3062-3069).
Refs: 45
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Caulobacter crescentus is a gram-negative ***bacterium*** that produces a two- dimensional crystalline array on its surface composed of a single 98-kDa protein, RsaA. Secretion of RsaA to the cell surface relies on an uncleaved C-terminal secretion signal. In this report, we identify two genes encoding components of the RsaA secretion apparatus. These components are part of a type I secretion system involving an ABC transporter protein. These genes, lying immediately 3' of rsaA, were found by screening a Tn5 transposon library for the loss of RsaA transport and characterizing the transposon- interrupted genes. The two proteins presumably encoded by these genes were found to have significant sequence similarity to ABC transporter and membrane ***fusion*** proteins of other type I secretion systems. The greatest sequence similarity was found to the alkaline protease (AprA) transport system of Pseudomonas aeruginosa and the metalloprotease (PrtB) transport system of Erwinia chrysanthemi. The prtB and aprA genes were introduced into C. crescentus, and their products were secreted by the RsaA transport system. Further, defects in the ***S*** - ***layer*** ***protein*** transport system led to the loss of this heterologous secretion. This is the first report of an ***S*** - ***layer*** ***protein*** secreted by a type I secretion apparatus. Unlike other type I secretion systems, the RsaA transport system secretes large amounts of its substrate protein (it is estimated that RsaA accounts for 10 to 12% of the total cell protein). Such levels are expected for ***bacterial*** S-layer proteins but are higher than for any other known type I secretion system.

L33 ANSWER 34 OF 66 MEDLINE
AN 1998194707 MEDLINE
DN 98194707 PubMed ID: 9535084
TI Serratia marcescens ***S*** - ***layer*** ***protein*** is secreted extracellularly via an ATP-binding cassette exporter, the Lip system.
AU Kawai E; Akatsuka H; Idei A; Shibatani T; Omori K
CS Lead Generation Research Laboratory, Tanabe Seiyaku Co., Ltd, Osaka, Japan.
SO MOLECULAR MICROBIOLOGY, (1998 Mar) 27 (5) 941-52.

Journal code: MOM; 8712028. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AB007124; GENBANK-AB007125
EM 199806
ED Entered STN: 19980618
Last Updated on STN: 20000303
Entered Medline: 19980609
AB The Serratia marcescens Lip exporter belonging to the ATP-binding cassette (ABC) exporter is known to be involved in signal peptide-independent extracellular secretion of a lipase and a metalloprotease. Although the genes of secretory proteins and their ABC exporters are usually all reported to be linked in several gram-negative ***bacteria*** , neither the lipase nor the protease gene is located close to the Lip exporter genes, lipBCD. A gene (slaA) located upstream of the lipBCD genes was cloned, revealing that it encodes a polypeptide of 100 kDa and is partially similar to the Caulobacter crescentus paracrystalline cell surface layer (***S*** - ***layer***) ***protein*** . The Lip exporter-deficient mutants of S. marcescens failed to secrete the SlaA protein. Electron micrography demonstrated the cell surface layer of S. marcescens. The ***S*** - ***layer*** ***protein*** was secreted to the cultured media in Escherichia coli cells carrying the Lip exporter. Three ABC exporters, Prt, Has and Hly systems, could not allow the S-layer secretion, indicating that the S. marcescens ***S*** - ***layer*** ***protein*** is strictly recognized by the Lip system. This is the first report concerning secretion of an ***S*** - ***layer*** ***protein*** via its own secretion system.

L33 ANSWER 35 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 19
AN 1998274055 EMBASE
TI A novel dipstick developed for rapid bet v 1-specific IgE detection: ***Recombinant*** allergen immobilized via a monoclonal antibody to crystalline ***bacterial*** cell-surface layers.
AU Breitwieser A.; Mader C.; Schocher I.; Hoffmann-Sommergruber K.; Aberer W.; Scheiner O.; Sleytr U.B.; Sara M.
CS Prof. M. Sara, Zentrum fur Ultrastrukturforschung, Universitat fur Bodenkultur, Gregor-Mendelstr. 33, 1180 Wien, Austria
SO Allergy: European Journal of Allergy and Clinical Immunology, (1998) 53/8 (786-793).
Refs: 25
ISSN: 0105-4538 CODEN: LLRGDY
CY Denmark
DT Journal; Article
FS 026 Immunology, Serology and Transplantation
LA English
SL English
AB The incidence of allergy to airborne proteins derived from tree and grass pollen, feces of mites, spores of molds, and pet dander has been increasing over the last decades. Since precise diagnosis is a prerequisite for successful immunotherapy, there is a rising demand for rapid, reliable, and inexpensive screening methods such as dipstick assays. With the purified ***recombinant*** major birch-pollen allergen rBet v 1a as model protein, crystalline ***bacterial***

cell-surface layers (S-layers) were tested for their applicability as an immobilization matrix for dipstick development. For this purpose, S-layers were deposited on a mechanically stable microporous support, cross-linked with glutaraldehyde, and free carboxylic acid groups of the ***S*** - ***layer*** ***protein*** were activated with carbodiimide. In the present test system, rBet v 1a was immobilized via the monoclonal mouse antibody BIP 1, which, unlike the allergen, is too large to enter the pores of the S-layer lattice, and which therefore formed a closed monolayer on the outermost surface of the crystal lattice. Moreover, BIP 1 is known to modulate IgE binding to the allergen. After incubation of the dipsticks in serum, washing of the reaction zone under tap water, and binding of an anti-IgE alkaline phosphatase conjugate, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium was used as substrate, forming an IgE concentration-dependent colored precipitate on the S-layer surface. The investigation of patient sera previously tested with the CAP(TM) system confirmed the specificity of the S- layer-based dipstick assay. Since the dipstick is easy to handle and the whole test procedure takes only 90 min, this test system should be applicable for rapid determination of specific IgE and for first screening in the doctor's practice.

L33 ANSWER 36 OF 66 MEDLINE
AN 1998195731 MEDLINE
DN 98195731 PubMed ID: 9534241
TI The S-layer gene of Lactobacillus helveticus CNRZ 892: cloning, sequence and heterologous expression.
AU Callegari M L; Riboli B; Sanders J W; Cocconcelli P S; Kok J; Venema G; Morelli L
CS Istituto di Microbiologia, Piacenza, Italy.. mcallega@cr.unicatt.it
SO MICROBIOLOGY, (1998 Mar) 144 (Pt 3) 719-26.
Journal code: BXW; 9430468. ISSN: 1350-0872.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AJ001931; GENBANK-X91199
EM 199805
ED Entered STN: 19980520
Last Updated on STN: 20000303
Entered Medline: 19980512
AB Lactobacillus helveticus CNRZ 892 contains a surface layer (S-layer) composed of protein monomers of 43 kDa organized in regular arrays. The gene encoding this protein (slpH) has been cloned in Escherichia coli and sequenced. slpH consists of 440 codons and is preceded by a ribosome-binding site (RBS) and followed by a putative rho-independent terminator. Indeed, Northern analysis revealed that slpH is a monocistronic gene. The gene is preceded by a possible promotor of which the -35 and -10 hexanucleotides are separated by 17 nt. By primer extension analysis the transcription start site was mapped at 7 nt downstream of the -10 sequence while the deduced amino acid sequence of SlpH shows a leader peptide of 30 aa. The slpH gene has been amplified by PCR and the fragment, carrying the complete gene from the RBS to the stop codon, has been cloned in a lactococcal gene expression vector downstream of promoter P32. Lactococcus lactis MG1363 carrying the resulting plasmid produced and secreted an S-layer monomer with the same molecular mass as

the authentic L. helveticus CNRZ 892 SlpH, as judged by SDS-PAGE. Immunoelectron microscopy revealed that SlpH was bound to the lactococcal cell walls in small clumps and accumulated in the growth medium as small sheets.

L33 ANSWER 37 OF 66 MEDLINE DUPLICATE 20
AN 1998442421 MEDLINE
DN 98442421 PubMed ID: 9770285
TI The ***S*** - ***layer*** ***protein*** from Campylobacter rectus: sequence determination and function of the ***recombinant*** protein.
AU Miyamoto M; Maeda H; Kitanaka M; Kokeguchi S; Takashiba S; Murayama Y
CS Department of Periodontology and Endodontology, Okayama University Dental School, Japan.
SO FEMS MICROBIOLOGY LETTERS, (1998 Sep 15) 166 (2) 275-81.
Journal code: FML; 7705721. ISSN: 0378-1097.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AB001876
EM 199811
ED Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981123
AB The gene encoding the crystalline surface layer (***S*** - ***layer***) ***protein*** from Campylobacter rectus, designated slp, was sequenced and the ***recombinant*** gene product was expressed in Escherichia coli. The gene consisted of 4086 nucleotides encoding a protein with 1361 amino acids. The N-terminal amino acid sequence revealed that Slp did not contain a signal sequence, but that the initial methionine residue was processed. The deduced amino acid sequence displayed some common characteristic features of S-layer proteins previously reported. A homology search showed a high similarity to the Campylobacter fetus S-layer proteins, especially in their N-terminus. The C-terminal third of Slp exhibited homology with the RTX toxins from Gram-negative ***bacteria*** via the region including the glycine-rich repeats. The Slp protein had the same N-terminal sequence as a 104-kDa cytotoxin isolated from the culture supernatants of C. rectus. However, neither native nor ***recombinant*** Slp showed cytotoxicity against HL-60 cells or human peripheral white blood cells. These data support the idea that the N-terminus acts as an anchor to the cell surface components and that the C-terminus is involved in the assembly and/or transport of the protein.

L33 ANSWER 38 OF 66 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD DUPLICATE 21
AN 1997-470880 [43] WPIDS
DNC C1997-149708
TI New DNA containing sequence for C-terminal region of Caulobacter ***S*** - ***layer*** ***protein*** - expressed as ***fusion*** proteins containing antigenic peptides in Caulobacter, useful as live vaccines.
DC B04 D15 D16 F09
IN BINGLE, W H; NOMELLINI, J F; SMIT, J

PA (UYBR-N) UNIV BRITISH COLUMBIA
CYC 24
PI WO 9734000 A1 19970918 (199743)* EN 58p
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU CA IL JP SG US
AU 9720194 A 19971001 (199805)
CA 2175549 A 19970913 (199815)
EP 888454 A1 19990107 (199906) EN
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
US 5976864 A 19991102 (199953)
JP 2000506020 W 20000523 (200033) 65p
AU 726384 B 20001102 (200062)
US 6210948 B1 20010403 (200120)
AU 2001018244 A 20010503 (200129)#
ADT WO 9734000 A1 WO 1997-CA167 19970310; AU 9720194 A AU 1997-20194 19970310; CA 2175549 A CA 1996-2175549 19960501; EP 888454 A1 EP 1997-908090 19970310, WO 1997-CA167 19970310; US 5976864 A CIP of US 1992-895367 19920609, CIP of US 1994-194290 19940209, US 1996-614377 19960312; JP 2000506020 W JP 1997-532138 19970310, WO 1997-CA167 19970310; AU 726384 B AU 1997-20194 19970310; US 6210948 B1 CIP of US 1992-895367 19920609, CIP of US 1994-194290 19940209, CIP of US 1996-614377 19960312, WO 1997-CA167 19970310, US 1999-142648 19990330; AU 2001018244 A Div ex AU 1997-20194 19970310, AU 2001-18244 20010202
FDT AU 9720194 A Based on WO 9734000; EP 888454 A1 Based on WO 9734000; US 5976864 A CIP of US 5500353; JP 2000506020 W Based on WO 9734000; AU 726384 B Previous Publ. AU 9720194, Based on WO 9734000; US 6210948 B1 CIP of US 5500353, CIP of US 5976864, Based on WO 9734000; AU 2001018244 A Div ex AU 726384
PRAI US 1996-614377 19960312; US 1992-895367 19920609; US 1994-194290 19940209; US 1999-142648 19990330; AU 2001-18244 20010202
AB WO 9734000 A UPAB: 19971030
A new DNA construct (I) contains at least one restriction site for insertion of DNA upstream of DNA (Ia) encoding a C-terminal region of at least the last 82 amino acids (aa) of Caulobacter ***S*** - ***layer*** ***protein*** (A). Also new are: (1) similar constructs (II) additionally containing a sequence (IIa) encoding a heterologous polypeptide (B), inserted upstream of, and in frame with, (Ia); (2) ***bacteria*** , specifically Caulobacter, containing (II); and (3) secreted proteins from the Caulobacter of (2) containing one or more (B) of up to 200 aa.

USE - Caulobacter containing (II) are particularly useful in live vaccines (where (B) is an antigen). They can also be used for production of e.g. ligands, enzymes or other proteins, e.g. metallothioneins to remove heavy metals from water or sewage, or xylanase or cellulase for use in wood pulping.

ADVANTAGE - All known Caulobacter strains are harmless, and stable in outdoor environments, including water (so suitable for vaccinating fish) or soil. They are well suited for growing in biofilm reactors and produce (A), which is an ideal system for presentation of antigens, at high level.
Dwg.5/12

L33 ANSWER 39 OF 66 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD DUPLICATE 22
AN 1997-394558 [37] WPIDS
DNC C1997-126945

TI Preparation of S-layer proteins by expressing sbs-A gene in Gram negative ***bacterium*** - or new sbs-B gene in any host, also new ***recombinant*** proteins containing heterologous inserts, e.g. epitope(s), useful as vaccines and adjuvants.
DC B04 C06 D16
IN KUEN, B; LUBITZ, W; SLEYTR, U; HOWORKA, S; RESCH, S; SARA, M; SCHROLL, G; TRUPPE, M
PA (LUBI-I) LUBITZ W; (SLEY-I) SLEYTR U; (SLEY-I) SLEYTR U B
CYC 75
PI DE 19603649 A1 19970807 (199737)* 31p
WO 9728263 A1 19970807 (199737) DE 69p
RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN
AU 9717203 A 19970822 (199801)
EP 882129 A1 19981209 (199902) DE
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
NZ 331300 A 19990629 (199931)
CN 1213402 A 19990407 (199932)
AU 713999 B 19991216 (200010)
JP 2000503850 W 20000404 (200027) 61p
ADT DE 19603649 A1 DE 1996-19603649 19960201; WO 9728263 A1 WO 1997-EP432 19970131; AU 9717203 A AU 1997-17203 19970131, WO 1997-EP432 19970131; EP 882129 A1 EP 1997-904360 19970131, WO 1997-EP432 19970131; NZ 331300 A NZ 1997-331300 19970131, WO 1997-EP432 19970131; CN 1213402 A CN 1997-192940 19970131; AU 713999 B AU 1997-17203 19970131; JP 2000503850 W JP 1997-527307 19970131, WO 1997-EP432 19970131
FDT AU 9717203 A Based on WO 9728263; EP 882129 A1 Based on WO 9728263; NZ 331300 A Based on WO 9728263; AU 713999 B Previous Publ. AU 9717203, Based on WO 9728263; JP 2000503850 W Based on WO 9728263
PRAI DE 1996-19603649 19960201
AB DE 19603649 A UPAB: 19970926
Preparation of ***S*** - ***layer*** ***protein*** (I), comprises transforming a Gram-negative prokaryotic host with a (I) encoding nucleic acid (II), and culturing the transformed cells.
USE - (I) and the S-layer structures are useful as vaccines or adjuvants, particularly when they include a ***bacterial*** ghost that may contain additional epitopes in its membrane. Other uses of ***recombinant*** (I), depending in the nature of the inserted peptide, are as an universal carrier for biotinylated reactants for use in immunological or hybridisation assays (the insert is streptavidin), to induce immune responses (epitopes), as a reagent for removing cytokine or toxin from serum (antigenic epitopes), as a molecular spinning nozzle (polyhydroxybutyrate synthase, PHBS) and as molecular laser (luciferase).
ADVANTAGE - When expressed in Gram-negative cells, (I) are produced in the form of monomolecular layers, rather than as inclusion bodies as in Gram-positive ***bacteria*** .
Dwg.2/3

L33 ANSWER 40 OF 66 CAPLUS COPYRIGHT 2001 ACS
AN 1997:536912 CAPLUS
DN 127:201021
TI Expression of S-layer proteins in Gram-negative ***bacteria*** and

recombinant chimeric S-layer proteins for use as vaccines
IN Lubitz, Werner; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
PA Lubitz, Werner, Austria; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
SO PCT Int. Appl., 65 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9728263	A1	19970807	WO 1997-EP432	19970131
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
DE 19603649	A1	19970807	DE 1996-19603649	19960201
CA 2245584	AA	19970807	CA 1997-2245584	19970131
AU 9717203	A1	19970822	AU 1997-17203	19970131
AU 713999	B2	19991216		
EP 882129	A1	19981209	EP 1997-904360	19970131
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
CN 1213402	A	19990407	CN 1997-192940	19970131
JP 2000503850	T2	20000404	JP 1997-527307	19970131
PRAI DE 1996-19603649		19960201		
WO 1997-EP432		19970131		

AB The invention concerns processess for the ***recombinant*** prepn. of S-layer proteins in Gram-neg. host cells. In addn., the nucleotide sequence of a new S-layer gene, the sbsB gene of Bacillus stearothermophilus, and a process for prepn. of modified S-layer proteins is disclosed. ***Recombinant*** Escherichia coli expressing the sbsA gene of B. stearothermophilus and chimeric sbsA genes encoding SbsA into which various peptides, proteins and enzymes have been inserted were prepd. and cultured to produce the proteins.

L33 ANSWER 41 OF 66 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1998:87452 BIOSIS
DN PREV199800087452
TI Bet v 1, the major birch pollen allergen, conjugated to crystalline ***bacterial*** cell surface proteins, expands allergen-specific T cells of the Th1/Th0 phenotype in vitro by induction of IL-12.
AU Jahn-Schmid, Beatrice; Siemann, Ute; Zenker, Andrea; Bohle, Barbara; Messner, Paul; Unger, Frank M.; Sleytr, Uwe B.; Scheiner, Otto; Kraft, Dietrich; Ebner, Christof (1)
CS (1) Inst. Allgemeine Experimentelle Pathologie, Univ. Wien, AKH-EWB-OST 3Q, Waehringer Guertel 18-20, 1090 Wien Austria
SO International Immunology, (Dec., 1997) Vol. 9, No. 12, pp. 1867-1874. ISSN: 0953-8178.
DT Article

LA English

AB Modulation of allergic immune responses by using adequate adjuvants is a promising concept for future immunotherapy of type I hypersensitivity. In the present study, ***recombinant*** Bet v 1 (rBet v 1, the major birch pollen allergen) was conjugated to cross-linked crystalline surface layer proteins (SL) derived from Gram-positive eubacteria. T cell lines (TCL) and clones (TCC) were established from peripheral blood of birch pollen-allergic patients. TCL and TCC were induced either using rBet v 1 alone or rBet v 1/SL conjugates (rBet v 1/SL) as initial antigen stimulus. Cytokine production after re-stimulation with rBet v 1 was investigated. TCL initiated with rBet v 1/SL showed significantly increased IFN-gamma production as compared to rBet v 1-selected TCL. TCC were established from TCL of five patients. As expected, the majority of CD4+ TCC induced by rBet v 1 (55%) displayed a Th2-like pattern of cytokine production. However, only 21% of Bet v 1 -specific TCC isolated from TCL established with the Bet v 1/SL revealed this phenotype. The majority of SL-specific TCC (80%) belonged to the Th1 phenotype. In cultures of peripheral blood mononuclear cells, both, SL and Bet v 1/SL (but not rBet v 1) stimulated the production of high levels of IL-12, a pivotal mediator of Th1 responses. Moreover, stimulation of rBet v 1-induced TCC with rBet v 1/SL led to an Increased IFN-gamma production. This effect could be reversed by neutralizing anti-IL-12 mAb. Together these results indicate an adjuvant effect of SL mediated by IL-12. Our results indicate that ***bacterial*** components, such as SL, displaying adjuvant effects may be suitable for immunotherapeutical vaccines for type I allergy.

L33 ANSWER 42 OF 66 MEDLINE DUPLICATE 23

AN 97158650 MEDLINE

DN 97158650 PubMed ID: 9006010

TI Linker mutagenesis of the Caulobacter crescentus ***S*** - ***layer*** ***protein*** : toward a definition of an N-terminal anchoring region and a C-terminal secretion signal and the potential for heterologous protein secretion.

AU Bingle W H; Nomellini J F; Smit J

CS Department of Microbiology and Immunology, The University of British Columbia, Vancouver, Canada.

SO JOURNAL OF BACTERIOLOGY, (1997 Feb) 179 (3) 601-11. Journal code: HH3; 2985120R. ISSN: 0021-9193.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199702

ED Entered STN: 19970313
Last Updated on STN: 19970313
Entered Medline: 19970228

AB Linker insertion mutagenesis was used to modify the paracrystalline surface layer (***S*** - ***layer***) ***protein*** (RsaA) of the gram-negative ***bacterium*** Caulobacter crescentus. Eleven unique BamHI linker insertions in the cloned rsaA gene were identified; at the protein level, these linker insertions introduced 4 to 6 amino acids at positions ranging from the extreme N terminus to the extreme C terminus of the 1,026-amino-acid RsaA protein. All linker-peptide insertions in the RsaA N terminus caused the secreted protein to be shed into the growth medium, suggesting that the RsaA N terminus is involved in cell surface

anchoring. One linker-peptide insertion in the RsaA C terminus (amino acid 784) had no effect on S-layer biogenesis, while another (amino acid 907) disrupted secretion of the protein, suggesting that RsaA possesses a secretion signal lying C terminal to amino acid 784, near or including amino acid 907. Unlike extreme N- or C-terminal linker-peptide insertions, those more centrally located in the RsaA primary sequence had no apparent effect on S-layer biogenesis. By using a newly introduced linker-encoded restriction site, a 3' fragment of the rsaA gene encoding the last 242 C-terminal amino acids of the ***S*** - ***layer*** ***protein*** was expressed in C. crescentus from heterologous Escherichia coli lacZ transcription and translation initiation information. This C-terminal portion of RsaA was secreted into the growth medium, confirming the presence of a C-terminal secretion signal. The use of the RsaA C terminus for the secretion of heterologous proteins in C. crescentus was explored by fusing 109 amino acids of an envelope glycoprotein from infectious hematopoietic necrosis virus, a pathogen of salmonid fish, to the last 242 amino acids of the RsaA C terminus. The resulting hybrid protein was successfully secreted into the growth medium and accounted for 10% of total protein in a stationary-phase culture. Based on these results and features of the RsaA primary sequence, we propose that the C. crescentus ***S*** - ***layer*** ***protein*** is secreted by a type I secretion system, relying on a stable C-terminal secretion signal in a manner analogous to E. coli alpha-hemolysin, the first example of an ***S*** - ***layer*** ***protein*** secreted by such a pathway.

L33 ANSWER 43 OF 66 MEDLINE DUPLICATE 24
AN 97228426 MEDLINE
DN 97228426 PubMed ID: 9074504
TI High level heterologous protein production in Lactococcus and Lactobacillus using a new secretion system based on the Lactobacillus brevis S-layer signals.
AU Savijoki K; Kahala M; Palva A
CS Agricultural Research Centre of Finland, Food Research Institute, Jokioinnen, Finland.
SO GENE, (1997 Feb 28) 186 (2) 255-62.
Journal code: FOP; 7706761. ISSN: 0378-1119.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199704
ED Entered STN: 19970424
Last Updated on STN: 19970424
Entered Medline: 19970414
AB A secretion cassette, based on the expression and secretion signals of a ***S*** - ***layer*** ***protein*** (SlpA) from Lactobacillus brevis, was constructed. E. coli beta-lactamase (Bla) was used as the reporter protein to determine the functionality of the S-layer signals for heterologous expression and secretion in Lactococcus lactis, Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus gasseri and Lactobacillus casei using a low-copy-number plasmid derived from pGK12. In all hosts tested, the bla gene was expressed under the slpA signals and all Bla activity was secreted to the culture medium. The Lb. brevis S-layer promoters were very efficiently recognized in L. lactis, Lb. brevis and Lb. plantarum, whereas in Lb. gasseri the slpA promoter region appeared to

be recognized at a lower level and in Lb. casei the level of transcripts was below the detection limit. The production of Bla was mainly restricted to the exponential phase of growth. The highest yield of Bla was obtained with L. lactis and Lb. brevis. Without pH control, substantial degradation of Bla occurred during prolonged cultivations with all lactic acid ***bacteria*** (LAB) tested. When growing L. lactis and Lb. brevis under pH control, the Bla activity could be stabilized also at the stationary phase. L. lactis produced up to 80 mg/l of Bla which to our knowledge represents the highest amount of a heterologous protein secreted by LAB so far. The short production phase implied a very high rate of secretion with a calculated value of 5 x 10(5) Bla molecules/cell per h. Such a high rate was also observed with Lb. plantarum, whereas in Lb. brevis the competition between the wild type slpA gene and the secretion construct probably lowered the rate of Bla production. The results obtained indicate wide applicability of the Lb. brevis slpA signals for efficient protein production and secretion in LAB.

L33 ANSWER 44 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 25
AN 97267948 EMBASE
DN 1997267948
TI IV. Molecular biology of S-layers.
AU Bahl H.; Scholz H.; Bayan N.; Chami M.; Leblon G.; Gulik-Krzywicki T.; Shechter E.; Fouet A.; Mesnage S.; Tosi-Couture E.; Gounon P.; Mock M.; De Macario E.C.; Macario A.J.L.; Fernandez-Herrero L.A.; Olabarria G.; Berenguer J.; Blaser M.J.; Kuen B.; Lubitz W.; Sara M.; Pouwels P.H.; Kolen C.P.A.M.; Boot H.J.; Palva A.; Truppe M.; Howorka S.; Schroll G.; Lechleitner S.; Resch S.
CS Dr. N. Bayan, Laboratoire des Biomembranes, URA 1116 CNRS, Universite de Paris-Sud, F-91405 Orsay, France
SO FEMS Microbiology Reviews, (1997) 20/1-2 (47-98).
Refs: 197
ISSN: 0168-6445 CODEN: FMREE4
PUI S 0168-6445(97)00050-8
CY Netherlands
DT Journal; General Review
FS 004 Microbiology
LA English
SL English
AB In this chapter we report on the molecular biology of crystalline surface layers of different ***bacterial*** groups. The limited information indicates that there are many variations on a common theme. Sequence variety, antigenic diversity, gene expression, rearrangements, influence of environmental factors and applied aspects are addressed. There is considerable variety in the S-layer composition, which was elucidated by sequence analysis of the corresponding genes. In Corynebacterium glutamicum one major cell wall protein is responsible for the formation of a highly ordered, hexagonal array. In contrast, two abundant surface proteins form the S-layer of Bacillus anthracis. Each protein possesses three S-layer homology motifs and one protein could be a virulence factor. The antigenic diversity and ABC transporters are important features, which have been studied in methanogenic archaea. The expression of the S-layer components is controlled by three genes in the case of Thermus thermophilus. One has repressor activity on the S-layer gene promoter, the second codes for the ***S*** - ***layer*** ***protein*** . The rearrangement by reciprocal recombination was investigated in

Campylobacter fetus. 7-8 S-layer proteins with a high degree of homology at the 5' and 3' ends were found. Environmental changes influence the surface properties of Bacillus stearothermophilus. Depending on oxygen supply, this species produces different S-layer proteins. Finaly, the molecular bases for some applications are discussed. ***Recombinant*** S-layer ***fusion*** proteins have been designed for biotechnology.

L33 ANSWER 45 OF 66 USPATFULL DUPLICATE 26
AN 96:23036 USPATFULL
TI ***Bacterial*** surface protein expression
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
PA The University of British Columbia, Vancouver, Canada (non-U.S. corporation)
PI US 5500353 19960319
AI US 1994-194290 19940209 (8)
RLI Continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Kim, Hyosuk
LREP Shlesinger, Arkwright & Garvey
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 898
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides a ***bacterium*** having an S-layer modified such that the ***bacterium*** ***S*** - ***layer*** ***protein*** gene contains one or more in-frame sequences coding for one or more heterologous polypeptides and, the S-layer is a ***fusion*** product of the ***S*** - ***layer*** ***protein*** and the heterologous polypeptide. The ***bacterium*** is preferably a Caulobacter which may be cultured as a film in a bioreactor or may be used to present an antigenic epitope to the environment of the ***bacterium*** . This invention also provides a method of expressing and presenting to the environment of a Caulobacter, a polypeptide that is heterologous to the S-layer of Caulobacter which comprises cloning a coding sequence for the polypeptide in-frame into an ***S*** - ***layer*** ***protein*** gene of Caulobacter whereby the polypeptide is expressed and presented on the surface of the Caulobacter as a ***fusion*** product of the ***S*** - ***layer*** ***protein*** and the polypeptide in the S-layer of the Caulobacter.

L33 ANSWER 46 OF 66 USPATFULL
AN 96:77808 USPATFULL
TI Methods for the synthesis of monofucosylated oligosaccharides terminating in di-N-acetyllactosaminyl structures
IN Kashem, Mohammed A., Edmonton, Canada
Venot, Andre P., Edmonton, Canada
Smith, Richard, Edmonton, Canada
PA Alberta Research Council, Alberta, Canada (non-U.S. corporation)
PI US 5550155 19960827
AI US 1994-323100 19941014 (8)
RLI Continuation of Ser. No. US 1992-914172, filed on 14 Jul 1992, now

patented, Pat. No. US 5374655 which is a continuation-in-part of Ser. No. US 1992-889017, filed on 26 May 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-771259, filed on 2 Oct 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-714161, filed on 10 Jun 1991
DT Utility
EXNAM Primary Examiner: Kight, III, John; Assistant Examiner: Leary, Louise N.
LREP Burns, Doane, Swecker & Mathis
CLMN Number of Claims: 7
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 1837
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Monofucosylated and monosialyated derivatives of the compound .beta.Gal(1-4).beta.GlcNAc(1-3).beta.Gal(1-4).beta.GlcNAc-OR, where R is hydrogen, a saccharide, an oligosaccharide or an aglycon moiety have been found to be useful in modulating a cell-mediated immune inflammatory response in mammals.

L33 ANSWER 47 OF 66 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1996-05577 BIOTECHDS
TI Nucleic acid encoding signal peptide of Bacillus stearothermophilus ***S*** - ***layer*** ***protein*** ;
for secretion of protein which has a high lysine content
AU Lubitz W
PA Vogelbusch
PI DE 4425527 25 Jan 1996
AI DE 1994-4425527 19 Jul 1994
PRAI DE 1994-4425527 19 Jul 1994
DT Patent
LA German
OS WPI: 1996-077933 [09]
AB A nucleic acid (I) encoding a functional signal peptide (SP) is new which is selected from: (a) the SP-encoding portion of a 3,706 bp sequence, (b) a sequence corresponding to (a) taking into account the degeneracy of the genetic code or (c) a sequence with at least 90% homology to (a) or (b). Also claimed are: (1) (I) operatively linked at its 3'-terminus to a protein-encoding nucleic acid, (2) (I) or the nucleic acid of (1) operatively linked at its 5'-terminus to an expression control sequence, (3) a protein encoded by a nucleic acid, (4) a ***recombinant*** vector containing at least 1 copy of a nucleic acid, (5) a host cell transformed with a nucleic acid or vector and (6) an expression control sequence. This process is useful for the production of Bacillus stearothermophlis ***S*** - ***layer*** ***protein*** , which has a lysine content of at least 10%. Optimally the protein in hydrolyzed and the amino acids recovered. (11pp)

L33 ANSWER 48 OF 66 MEDLINE
AN 96349118 MEDLINE
DN 96349118 PubMed ID: 8760925
TI SomA, a novel gene that encodes a major outer-membrane protein of Synechococcus sp. PCC 7942.
AU Umeda H; Aiba H; Mizuno T
CS Laboratory of Molecular Microbiology, School of Agriculture, Nagoya University, Japan.

SO MICROBIOLOGY, (1996 Aug) 142 (Pt 8) 2121-8.
Journal code: BXW; 9430468. ISSN: 1350-0872.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-D64077
EM 199609
ED Entered STN: 19961015
Last Updated on STN: 19961015
Entered Medline: 19960930
AB The outer membrane of a cyanobacterium (Synechococcus sp. strain PCC 7942) contains only a few major proteins. A gene encoding one of them, somA, was cloned and characterized. Based on the nucleotide sequence, SomA was predicted to comprise 531 amino acids with a calculated molecular mass of 57,136 Da. The deduced amino acid sequence of SomA shares similarities with two ***bacterial*** cell-surface proteins, the ***S*** - ***layer*** ***protein*** of Thermus thermophilus and the flagellin of Campylobacter coli. The predicted amino acid sequence of SomA revealed also that it contains a signal peptide-like sequence at its N terminus. This signal peptide-like sequence was capable of mediating protein translocation across the cytoplasmic membrane into the outer membrane of Escherichia coli, provided that this sequence was fused to the E. coli outer-membrane protein, OmpF. The signal peptide-like sequence was cleaved upon the translocation of the SomA::OmpF protein. We suggest that SomA is synthesized as a precursor and that its N-terminal 24 amino acid sequence is a cleavable signal peptide involved in protein targeting into the outer membrane. To our knowledge, this is the first example of cleavable signal peptides for proteins transported into the outer membrane of cyanobacteria.

L33 ANSWER 49 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 27
AN 96346348 EMBASE
DN 1996346348
TI 2-D protein crystals as an immobilization matrix for producing reaction zones in dipstick-style immunoassays.
AU Breitwieser A.; Kupcu S.; Howorka S.; Weiger S.; Langer C.; Hoffmann-Sommergruber K.; Scheiner O.; Sleytr U.B.; Sara M.
CS ZULB, Inst. fur Molekulare Nanotechnologie, Universitat fur Bodenkultur, Gregor Mendelstrasse 33,A-1180 Vienna, Austria
SO BioTechniques, (1996) 21/5 (918-925).
ISSN: 0736-6205 CODEN: BTNQDO
CY United States
DT Journal; Article
FS 004 Microbiology
027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
LA English
SL English
AB In the present study, the applicability of crystalline ***bacterial*** cell- surface layers (S-layers) as novel immobilization matrices and reaction zones for dipstick-style immunoassays was investigated. For this purpose, S-layer- carrying cell-wall fragments from Bacillus sphaericus CCM 2120 were deposited on a microporous support, and the ***S*** - ***layer*** ***protein*** was cross-linked with glutaraldehyde. For

developing appropriate test systems, either human IgG was directly linked to the carboxylic acid groups from the ***S*** - ***layer*** ***protein*** or it was immobilized using Protein A or, after biotinylation, using streptavidin. A clear correlation was obtained between the amount of anti-human IgG applied and the absorbance values in the immunoassays. S-layers with covalently bound ***recombinant*** major birch pollen allergen were used for quantitative and semiquantitative determination of an antibody raised against it. Using S-layers as an immobilization matrix in comparison to amorphous polymers has advantages in that the closed monolayers of functional macromolecules on their outermost surface allows for strong signals in immunoassays, almost completely eliminates background and prevents diffusion.

L33 ANSWER 50 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 28
AN 96269060 EMBASE
DN 1996269060
TI The extreme N-terminus of the Caulobacter crescentus surface-layer protein directs export of passenger proteins from the cytoplasm but is not required for secretion of the native protein.
AU Bingle W.H.; Le K.D.; Smit J.
CS Microbiology/Immunology Department, University of British Columbia,Vancouver, BC V6T 1Z3, Canada
SO Canadian Journal of Microbiology, (1996) 42/7 (672-684).
ISSN: 0008-4166 CODEN: CJMIAZ
CY Canada
DT Journal; Article
FS 004 Microbiology
LA English
SL English; French
AB The paracrystalline surface layer (S-layer) of Caulobacter crescentus is composed era single protein (RsaA, 1026 amino acids) that associates noncovalently with the lipopolysaccharide of the outer membrane. Like many other extracellular proteins of Gram-negative ***bacteria*** , the ***S*** - ***layer*** ***protein*** is not processed during transport to the cell surface. To study the secretion of RsaA, several N-terminal deletions of the protein were made by modifying the 5'-region of the rsaA gene. This analysis showed that portions of the N-terminus totalling the first 775 N-terminal amino acids (75% of the protein) could be removed from RsaA without abolishing secretion of the remainder of the protein. Although the RsaA N-terminus was not required for secretion, an N-terminal domain consisting of either 34 or 52 RsaA-derived amino acids promoted export of the alkaline phosphatase reporter (PhoA) and a cellulase reporter (.DELTA.CenA) from the cytoplasm: using the cellulase reporter, the efficiency of hybrid protein export was estimated at 9%. No enzyme activity was detected in the cell-free culture fluids as the result of expressing any gene ***fusion*** , indicating that no hybrid protein was completely secreted from the cell. RsaA:PhoA hybrid proteins were also exported from the E. coli cytoplasm, a ***bacterium*** not expected to contain the necessary machinery for the secretion of RsaA. Taken together, these data indicate that the secretion pathway of RsaA relies on a C-terminal secretion signal and that once separated from the content of the native protein, the extreme N-terminus of RsaA can act as an inefficient cryptic export signal that is not used during native RsaA secretion.

L33 ANSWER 51 OF 66 MEDLINE
AN 96228698 MEDLINE
DN 96228698 PubMed ID: 8830240
TI Heterologous expression and self-assembly of the ***S*** - ***layer*** ***protein*** SbsA of Bacillus stearothermophilus in Escherichia coli.
AU Kuen B; Sara M; Lubitz W
CS Institut fur Mikrobiologie und Genetik, Universitat, Austria.. oetzi@gem.univie.ac.at
SO MOLECULAR MICROBIOLOGY, (1996 Feb) 19 (3) 495-503.
Journal code: MOM; 8712028. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199610
ED Entered STN: 19961025
Last Updated on STN: 19961025
Entered Medline: 19961016
AB The cell surface of Bacillus stearothermophilus PV72 is covered by a regular surface layer (S-layer) composed of single species of protein, SbsA, with a molecular weight of 130,000. Recently, the sequence of the corresponding gene (sbsA) has been determined. The SbsA coding region including the signal sequence was cloned as a polymerase chain reaction (PCR) product into a low-copy-number vector under the transcriptional control of the lambda pL promoter. Expression of sbsA was shown to be thermally inducible from the resulting vector pBK4 in a strain of Escherichia coli expressing the lambda cI857 from the chromosome. As shown by ultrathin sectioning of whole cells and immunogold labelling using SbsA- specific antibodies, expression of sbsA in E. coli led to accumulation of sheet-like self-assembling products of the protein in the cytoplasm. No SbsA protein was detected either in the periplasm or in the supernatant fractions. Long-term expression of sbsA from pBK4, including in the late stationary phase, did not lead to degradation of SbsA.

L33 ANSWER 52 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 29
AN 96100429 EMBASE
DN 1996100429
TI Expression and purification of the crystalline surface layer protein of Rickettsia typhi.
AU Hahn M.-J.; Chang W.-H.
CS Department of Microbiology, College of Medicine, Kon-Kuk University,Danwol-Dong, Choongju 380-701, Korea, Republic of
SO Microbiology and Immunology, (1996) 40/3 (233-236).
ISSN: 0385-5600 CODEN: MIIMDV
CY Japan
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The crystalline surface layer (***S*** - ***layer*** ***protein*** (SLP) of Rickettsia typhi is known as the protective antigen against murine typhus. We previously reported a cloning and sequence analysis of the SLP gene of R. typhi (slpT) and showed that the open reading frame of this gene encodes both the SLP and a 32-kDa protein. To express only the SLP from this gene, the putative signal sequence and

the 32-kDa protein portion were removed from the slpT. This protein was expressed in Escherichia coli as a ***fusion*** protein, consisting of the SLP and maltose binding protein. The ***recombinant*** protein reacted strongly with polyclonal antiserum of a patient with murine typhus.

L33 ANSWER 53 OF 66 JICST-EPlus COPYRIGHT 2001 JST
AN 960584091 JICST-EPlus
TI Characterzation of ***Recombinant*** 150-kDa ***S*** - ***layer*** ***Protein*** from Campylobacter rectus.
AU HIRATSUKA K; HAYAKAWA M; TAKIGUCHI H; ABIKO Y
CS Nihon Univ. School of Dentistry at Matsudo, Chiba, JPN
SO Nichidai Koku Kagaku (Nihon University Journal of Oral Science), (1996) vol. 22, no. 2, pp. 139-148. Journal Code: Z0705A (Fig. 6, Tbl. 1, Ref. 29)
ISSN: 0385-0145
CY Japan
DT Journal; Article
LA English
STA New
AB The gene encoding the cell surface layer (S-layer) antigen of molecular mass of 150-kDa was isolated from a genomic library of Campylobacter rectus ATCC 33238 constructed in .LAMBDA. GEM-11 by screening with an antiserum against ***bacterial*** cell sonicates. This gene was further subcloned into the plasmid vector pACYC184 and expressed in Escherichia coli. The purified 150-kDa ***recombinant*** protein was charaterized. Western immunoblotting showed that the 150-kDa protein was expressed in E. coli and that the anti- ***recombinant*** protein serum reacted with native 150-kDa antigen of C. rectus. Amino acid sequencing revealed perfect agreement at the N-terminus between the native S-layer antigen and the ***recombinant*** protein. Immunocytochemical electron microscopy using an affinity-purified antibody against the ***recombinant*** protein revealed the 150-kDa antigen on the C. rectus cell surface. The recombinsmt protein was strain-specific for C. rectus according to Westernblotting analysis. The immunoreactivities of the sera from human to the ***recombinant*** 150-kDa antigen were analysed by Western immunoblotting analysis. The sera of some patients with gingivitis and periodontitis reacted strongly, whereas the sera of children showed no reactivity in responce to the 150-kDa ***recombinant*** antigen. These data suggested that the gene encoding the C. rectus 150-kDa ***S*** - ***layer*** ***protein*** was cloned and functionally expressed in E. coli., and the ***recombinant*** 150-kDa protein was useful for serological diagnosis as the C. rectus specific antigen. (author abst.)

L33 ANSWER 54 OF 66 LIFESCI COPYRIGHT 2001 CSA
AN 97:61228 LIFESCI
TI ***Bacterial*** surface protein expression
CS UNIVERSITY OF BRITISH COLUMBIA
SO (1996) . US Patent 5500353; US Cl. 435/69.1 424/192.1 424/197.11 435/69.3 435/69.7 435/177 435/209 435/252.3 514/6 530/350 530/395 530/400 536/22.1 536/23.1 536/23.4 536/23.7.
DT Patent
FS W2; A
LA English
AB This invention provides a ***bacterium*** having an S-layer modified

such that the ***bacterium*** ***S*** - ***layer*** ***protein*** gene contains one or more in-frame sequences coding for one or more heterologous polypeptides and, the S-layer is a ***fusion*** product of the ***S*** - ***layer*** ***protein*** and the heterologous polypeptide. The ***bacterium*** is preferably a Caulobacter which may be cultured as a film in a bioreactor or may be used to present an antigenic epitope to the environment of the ***bacterium*** . This invention also provides a method of expressing and presenting to the environment of a Caulobacter, a polypeptide that is heterologous to the S-layer of Caulobacter which comprises cloning a coding sequence for the polypeptide in-frame into an ***S*** - ***layer*** ***protein*** gene of Caulobacter whereby the polypeptide is expressed and presented on the surface of the Caulobacter as a ***fusion*** product of the ***S*** - ***layer*** ***protein*** and the polypeptide in the S-layer of the Caulobacter.

L33 ANSWER 55 OF 66 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1995-11949 BIOTECHDS
TI Host cell expressing surface layer protein; Bordetella pertussis P69 antigen, pertussis toxin, tetanus toxin fragment-C, Escherichia coli heat-labile toxin B-subunit or E. coli K88 antigen surface display on Bacillus sphaericus
AU Deblaere R Y; Desomer J; Dhaese P
PA Solvay
PI WO 9519371 20 Jul 1995
AI WO 1995-EP147 13 Jan 1995
PRAI GB 1994-650 14 Jan 1994
DT Patent
LA English
OS WPI: 1995-263827 [34]
AB A new host cell has a surface layer (S-layer) containing a ***fusion*** protein, composed of at least sufficient ***S*** - ***layer*** ***protein*** for assembly, and a heterologous protein fragment fused to the C-terminus or N-terminus, which is then presented on the outer surface of the cell. The following are also new: DNA containing a promoter (e.g. a Bacillus sp. ***S*** - ***layer*** ***protein*** promoter, such as the P1 promoter of Bacillus sphaericus P-1 (LMG P-13855)) operably linked to a sequence encoding a signal peptide and the ***fusion*** protein; a promoter with specified -35 and -10 regions; an expression vector with the promoter and a downstream cloning site; and a process for transformation of B. sphaericus P-1 by harvesting cells at late stationary phase, mixing with DNA, and carrying out electroporation. The heterologous protein may be a virus, ***bacterium*** , fungus, yeast or parasite antigen, e.g. Bordetella pertussis P69 antigen, pertussis toxin or a subunit, tetanus toxin fragment-C, Escherichia coli heat-labile toxin B-subunit or E. coli K88 antigen. Cells presenting the ***fusion*** protein on their surface may be used as a ***recombinant*** vaccine. (95pp)

L33 ANSWER 56 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 30
AN 95190761 EMBASE
DN 1995190761
TI Segmental conservation of sapA sequences in type B Campylobacter fetus cells.
AU Dworkin J.; Tummuru M.K.R.; Blaser M.J.

CS Division of Infectious Diseases, A-3310 Medical Center North, Vanderbilt Univ. School of Medicine,Nashville, TN 37232-2605, United States
SO Journal of Biological Chemistry, (1995) 270/25 (15093-15101). ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal; Article
FS 004 Microbiology
029 Clinical Biochemistry
LA English
SL English
AB Campylobacter fetus cells may exist as either of two defined serogroups (type A or B) based on their lipopolysaccharide (LPS) composition. Wild-type swains contain surface array proteins (S-layer proteins) that have partial antigenic cross-reactivity but bind exclusively to LPS from homologous (type A or B) cells. Type A cells possess 8 homologs of sapA, which encodes a 97- kDa ***S*** - ***layer*** ***protein*** ; the gene products of these homologs have a conserved N terminus of 184 amino acids. To further explore the structural relationships between the C. fetus S-layer proteins and their encoding genes, we sought to clone and express an ***S*** - ***layer*** ***protein*** from type B strain 84-91. The cloned type B gene (sapB) was similar in structure to the previously cloned type A gene (sapA) and encoded a full-length 936-amino acid (97-kDa) ***S*** - ***layer*** ***protein*** . Sequence analysis of sapB indicated that the conserved N-terminal encoding region in sapA was absent but that the remainder of the ORF (encoding 751 amino acids) was identical to that of sapA in spite of the nonconserved nature of this region among sapA homologs. Noncoding sequences hath 300 base pairs 5' and 1000 base pairs 3' to the sapB and sapA ORFs, including the sapA promoter and transcriptional terminator sequences, were essentially identical. Southern analyses revealed that the sapB N-terminal encoding region was conserved in multiple copies in type B strains but was absent in type A strains. ***Recombinant*** sapA and sapB products bound to a substantially greater degree to cells of the homologous LPS type compared with the heterologous LPS type, indicating that the conserved sapA- and sapB- encoded N termini are critical for LPS binding specificity. The parallel genetic organization and identity at the nucleotide level in both coding and noncoding regions for sap homologs in types A and B cells indicates the necessity of both homolog conservation and high fidelity DNA replication in the biology of sap diversity.

L33 ANSWER 57 OF 66 USPATFULL
AN 94:110797 USPATFULL
TI Methods for the synthesis of monofucosylated oligosaccharides terminating in di-N-acetyllactosaminyl structures
IN Kashem, Mohammed, Edmonton, Canada
Venot, Andre P., Edmonton, Canada
Smith, Richard, Edmonton, Canada
PA Alberta Research Council, Edmonton, Canada (non-U.S. corporation)
PI US 5374655 19941220
AI US 1992-914172 19920714 (7)
RLI Continuation-in-part of Ser. No. US 1992-889017, filed on 26 May 1992 which is a continuation-in-part of Ser. No. US 1991-771259, filed on 2 Oct 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-714161, filed on 10 Jun 1991
PRAI WO 1992-251 19920610

DT Utility
EXNAM Primary Examiner: Russel, Jeffrey E.; Assistant Examiner: Leary, Louise N.
LREP Burns, Doane, Swecker & Mathis
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 2027
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are methods for the preparation of monofucosylated and sialylated derivatives of the compound .beta.Gal(1-4).beta.GlcNAc(1-3).beta.Gal(1-4).beta.GlcNAc-OR. In particular, the methods of this invention provide for a multi-step synthesis wherein selective monofucosylation is accomplished on the 3-hydroxy group on only one of the GlcNAc units found in the .beta.Gal(1-4) .beta.GlcNAc (1-3) .beta.Gal (1-4) .beta.GlcNAc-OR compound. In this step, monofucosylation is achieved by use of the .alpha.(1-3)fucosyltransferase.

L33 ANSWER 58 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 31
AN 94321308 EMBASE
DN 1994321308
TI Alkaline phosphatase and a cellulase reporter protein are not exported from the cytoplasm when fused to large N-terminal portions of the Caulobacter crescentus surface (***S***)- ***layer*** ***protein*** .
AU Bingle W.H.; Smit J.
CS Department Microbiology/Immunology, University of British Columbia,Vancouver, BC V6T 1Z3, Canada
SO Canadian Journal of Microbiology, (1994) 40/9 (777-782).
ISSN: 0008-4166 CODEN: CJMIAZ
CY Canada
DT Journal; Article
FS 004 Microbiology
LA English
SL English; French
AB Using a gene ***fusion*** approach, hybrid proteins were created by linking alkaline phosphatase (PhoA) or a cellulase reporter (.DELTA.CenA) to four large N-terminal portions of the Caulobacter crescentus surface (***S***)- ***layer*** ***protein*** (RsaA; 1026 amino acids). Three of the sites (aminoacids 189, 220, 315) were selected on the basis of TnphoA experiments that suggested the first 250-350 amino acids of RsaA could mediate export of PhoA from the cytoplasm while the fourth lay only 21 amino acids from the C-terminus. Expression of all fusions except rsaA(315):.DELTA.cenA and rsaA(315):phoA was toxic to C. crescentus. None of the gene fusions were toxic when expressed by Escherichia coli DH5.alpha., where all the hybrid proteins accumulated as inclusion bodies. The toxicity of hybrid proteins encoding 189, 220, and 1005 RsaA-derived amino acids was related to the nature of the hybrid protein itself because truncated RsaA peptides lacking their reporter domains were nontoxic. Further study of RsaA(.DELTA.C21) showed that this and presumably other truncated RsaA derivatives were neither secreted nor prone to intracellular accumulation. Although C. crescentus tolerated the expression of rsaA(315):.DELTA.cenA and rsaA(315):phoA, the encoded hybrid proteins were not exported in significant quantities from the cytoplasm. These results extend and confirm earlier work that large portions of the

S - ***layer*** ***protein*** N-terminus cannot mediate export of passenger proteins from the cytoplasm and that the entire native ***S*** - ***layer*** ***protein*** may be required to properly interact with the RsaA secretion machinery.

L33 ANSWER 59 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 32
AN 94059717 EMBASE
DN 1994059717
TI Molecular cloning of the ***S*** - ***layer*** ***protein*** gene of Campylobacter rectus ATCC 33238.
AU Miyamoto M.; Kobayashi Y.; Kokeguchi S.; Ohta H.; Kurihara H.; Fukui K.; Murayama Y.
CS Periodontology/Endodontology Dept., Okayama University Dental School, 2-5-1 Shikata-cho,Okayama 700, Japan
SO FEMS Microbiology Letters, (1994) 116/1 (13-18).
ISSN: 0378-1097 CODEN: FMLED7
CY Netherlands
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB A genomic DNA library of Campylobacter rectus (Wolinella recta) ATCC 33238 was constructed using ***bacteriophage*** lambha EMBL3 as a vector. One clone expressing the ***S*** - ***layer*** ***protein*** was identified immunologically with the antiserum to the ***S*** - ***layer*** ***protein*** of C. rectus ATCC 33238. Western immunoblotting using monoclonal antibodies directed against the ***S*** - ***layer*** ***protein*** showed a single blot of ***recombinant*** protein at 150 kDa, suggesting that this clone contained the entire coding region of the ***S*** - ***layer*** ***protein*** gene. Additionally, the ***S*** - ***layer*** ***protein*** gene was subcloned into plasmid vector pUC18. Southern hybridization revealed that the ***S*** - ***layer*** ***protein*** gene was present on the chromosome of C. rectus as a single-copy gene, and that there were minor heterogeneities among the ***S*** - ***layer*** ***protein*** genes of clinical isolates. Moreover, a spontaneous stable mutant strain, which has no S-layer expression, also conserved the full-length gene of the ***S*** - ***layer*** ***protein*** .

L33 ANSWER 60 OF 66 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD DUPLICATE 33
AN 1994-066249 [09] WPIDS
CR 1996-171046 [17]
DNC C1994-029749
TI Prodn. of heterologous polypeptides in ***bacteria*** , partic. Caulobacter - by expression of a ***fusion*** prod. of the polypeptide sequence and a ***bacterial*** ***S*** - ***layer*** ***protein*** gene.
DC B04 C06 D15 D16
IN BINGLE, W H; SMIT, J
PA (UYBR-N) UNIV BRITISH COLUMBIA
CYC 1
PI CA 2090549 A 19931210 (199409)* 27p
ADT CA 2090549 A CA 1993-2090549 19930226

PRAI US 1992-895367 19920609
AB CA 2090549 A UPAB: 19960503
A novel ***bacterium*** has an S-layer, where the ***bacterium*** 's ***S*** - ***layer*** ***protein*** gene contains one or more sequences encoding one or more functional heterologous polypeptides and the S-layer is a ***fusion*** prod. of the ***S*** - ***layer*** ***protein*** and the heterologous polypeptides. The heterologous polypeptide may be e.g. cellulase, xylanase or a metallothionein.

USE/ADVANTAGE - The ***S*** - ***layer*** ***protein*** ***bacterial*** system can be used in bioreactors, e.g. to bind toxic metals in sewage, waste water etc., or for treatment of wood pulp suspensions. The system can also be used to produce heterologous proteins at the organisms cell surface for use in vaccines, partic. fish vaccines.

The ***S*** - ***layer*** ***protein*** is synthesised in large quantities and has a generally repetitive sequence, permitting the synthesis of large amts. of heterologous protein as a ***fusion*** prod. and presentation at the cell surface.

In an example, the rsa A (S-layer) gene of C-crescentus was provided as pTZ188U:ras A delta P. Using Taq I, a partial digestion of the rsaA gene in pTZ18U:rsa A delta P produced a gp. of linearised segments with random Taq I sites cleaved. The linearised segments were modified by use of a tagged linker nutagenesis procedure using a 126p BamHI linker carried in puc102k.

Those prods. that produced a full-length protein in E.coli were transferred to pWB1 and introduced into C.crescentus. The transformants were analysed for the ability to produce a full-length protein in C.crescentus and to produce the crystalline S-layer on their surface. A Taq I site corresponding to amino acid 188 was identified. The coding sequence of monkey metallothionein II peptide was introduced into this site and expressed to produce a prod. which bound cadmium and copper.
Dwg.0/6
Dwg.0/6

L33 ANSWER 61 OF 66 MEDLINE
AN 94018618 MEDLINE
DN 94018618 PubMed ID: 8412676
TI Characterization of the cspB gene encoding PS2, an ordered surface-layer protein in Corynebacterium glutamicum.
AU Peyret J L; Bayan N; Joliff G; Gulik-Krzywicki T; Mathieu L; Schechter E; Leblon G
CS Laboratoire de Biologie Moleculaire des Coryne-bacteries, URA D1354 CNRS et GDR 961, Universite Paris-Sud, Orsay, France.
SO MOLECULAR MICROBIOLOGY, (1993 Jul) 9 (1) 97-109.
Journal code: MOM; 8712028. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-X69103
EM 199311
ED Entered STN: 19940117
Last Updated on STN: 19940117
Entered Medline: 19931104
AB PS2 is one of two major proteins detected in the culture media of various Corynebacterium glutamicum strains. The coding and promoter regions of the

cspB gene encoding PS2 were cloned in lambda gt11 using polyclonal antibodies raised against PS2 for screening. Expression of the cspB gene in Escherichia coli led to the production of a major anti-PS2 labelled peptide of 63,000 Da, corresponding presumably to the mature form of PS2. It was detected in the cytoplasm, periplasm and surrounding medium of E. coli. Three other slower migrating bands of 65,000 68,000 and 72,000 Da were detected. The largest one probably corresponds to the precursor form of PS2 in E. coli. Analysis of the nucleotide sequence revealed an open reading frame (ORF) of 1533 nucleotides. The deduced 510-amino-acid polypeptide had a calculated molecular mass of 55,426 Da. According to the predicted amino acid sequence, PS2 is synthesized with a N-terminal segment of 30-amino-acid residues reminiscent of eukaryotic and prokaryotic signal peptides, and a hydrophobic domain of 21 residues near the C-terminus. Although no significant homologies were found with other proteins, it appears that some characteristics and the amino acid composition of PS2 share several common features with surface-layer proteins. The cspB gene was then disrupted in C. glutamicum by gene replacement. Freeze-etching electron microscopy performed on the wild-type strain indicated that the cell wall of C. glutamicum is covered with an ordered surface of proteins (surface layer, S-layer) which is in very close contact with other cell-wall components. These structures are absent from the cspB-disrupted strain but are present after reintroduction of the cspB gene on a plasmid into this mutant. Thus we demonstrate that the ***S*** - ***layer*** ***protein*** is the product of the cspB gene.

L33 ANSWER 62 OF 66 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 34
AN 93098427 EMBASE
DN 1993098427
TI An 'all-purpose' cellulase reporter for gene ***fusion*** studies and application to the paracrystalline surface (***S***)- ***layer*** ***protein*** of Caulobacter crescentus.
AU Bingle W.H.; Kurtz Jr. H.D.; Smit J.
CS Department of Microbiology, University of British Columbia,Vancouver, BC V6T 1Z3, Canada
SO Canadian Journal of Microbiology, (1993) 39/1 (70-80).
ISSN: 0008-4166 CODEN: CJMIAZ
CY Canada
DT Journal; Article
FS 004 Microbiology
LA English
SL English; French
AB The secreted endoglucanase (CenA) from the Gram-positive ***bacterium*** Cellulomonas fimi and a deletion derivative (.DELTA.CenA) lacking the N-terminal leader peptide of native CenA were used to explore the potential of .DELTA.CenA as a reporter molecule in Caulobacter crescentus. Expression of cenA in C. crescentus yielded extracellular endoglucanase activity; suggesting that the N-terminal leader peptide of CenA could direct the enzyme to the periplasm where it subsequently leaked into the medium. In contrast, expression of .DELTA.cenA yielded only cell-associated endoglucanase activity; this suggested that the enzyme retained activity in the C. crescentus cytoplasm. Using the putative cytoplasmic and periplasmic forms of .DELTA.CenA as markers, a simple assay for periplasmic .DELTA.CenA hybrids was developed. This assay indicated that .DELTA.CenA activity was largely independent of cellular

location. To facilitate the use of .DELTA.CenA as a reporter, a broad host range translational ***fusion*** vector (pEC215) incorporating .DELTA.cenA was constructed. This vector was used to investigate factors important to the expression of the gene (rsaA) encoding the paracrystalline surface protein (S-layer) of the ***bacterium*** . It was found that altering the 5' untranslated region of the rsaA mRNA reduced gene expression by 70%. One rsaA:.DELTA.cenA gene ***fusion*** resulting from these experiments that incorporated only rsaA translation initiation information was further modified to serve as a general reporter for creating transcriptional gene fusions with other promoters. Gene fusions between alkaline phosphatase (phoA) and either cenA or lacZ were used to supplement information about RsaA secretion derived from rsaA:phoA gene fusions. It was found that linkage of the N-terminal leader peptide of CenA to PhoA yielded 50-100 times more cell-associated PhoA activity in C. crescentus than linkage of the RsaA N terminus. Taken together, these experiments indicated that .DELTA.CenA was useful for tagging proteins localized to the cytoplasm, exported to the periplasm, or secreted from the cell, as well as for monitoring events in the cytoplasm such as examining factors important to the level of gene expression. Further, because .DELTA.CenA was active in all cell compartments, it could be used to estimate the efficiency of hybrid protein export-secretion from enzyme activity measurements alone. In short, .DELTA.CenA possessed many of the attributes of an 'all-purpose' reporter.

L33 ANSWER 63 OF 66 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1993-04877 BIOTECHDS
TI Characterization of expression and secretion signals from Lactobacillus brevis for use in food and feed industry; characterization of the ***S*** - ***layer*** ***protein*** gene of potential use in a gene expression and ***recombinant*** protein secretion DNA cassette (conference paper)
AU Palva A; Vidgren G; Palva I; Pakkanen R; Lounatmaa K
CS Valio-Bioprod.
LO Agricultural Research Centre of Finland, Food Research Institute, 31600 Jokioinen, Finland.
SO Meded.Fac.Landbouwwet.Rijksuniv.Gent; (1992) 57, 4b, 1891-98
CODEN: MFLRA3
DT Journal
LA English
AB The ***S*** - ***layer*** ***protein*** gene of Lactobacillus brevis was cloned using the polymerase chain reaction with degenerated primers synthesized on the basis of the N-terminal amino acid sequence of the protein. PCR fragments containing the entire S-layer gene and its regulatory regions were sequenced to reveal an open reading frame with a coding capacity of 48,159 Da. From the regulatory region of the gene, tandem promoters and a ribosome binding site were found that showed typical features of prokaryotic consensus sequences. The coding region included a signal peptide of 30 amino acids, removal of the which resulted in a protein of 435 amino acids that corresponded in size to the ***S*** - ***layer*** ***protein*** determined by SDS-PAGE. The size and the 5' end analyses of the S-layer transcripts confirmed the monocistronic nature of the S-layer operon and the functionality of the 2 promoters found. The L. brevis S-layer gene hybridized with chromosomal DNA from Lactobacillus buchneri. Preliminary data showed that a gene expression-protein secretion cassette based on the L. brevis S-layer gene

was functional in Lactobacillus casei and Lactococcus lactis. (22 ref)

L33 ANSWER 64 OF 66 MEDLINE
AN 92138618 MEDLINE
DN 92138618 PubMed ID: 1735716
TI Reattachment of surface array proteins to Campylobacter fetus cells.
AU Yang L Y; Pei Z H; Fujimoto S; Blaser M J
CS Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.
NC R01AI-24145 (NIAID)
SO JOURNAL OF BACTERIOLOGY, (1992 Feb) 174 (4) 1258-67.
Journal code: HH3; 2985120R. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199203
ED Entered STN: 19920329
Last Updated on STN: 19920329
Entered Medline: 19920310
AB Campylobacter fetus strains may be of serotype A or B, a property associated with lipopolysaccharide (LPS) structure. Wild-type C. fetus strains contain surface array proteins (S-layer proteins) that may be extracted in water and that are critical for virulence. To explore the relationship of S-layer proteins to other surface components, we reattached S-layer proteins onto S- template cells generated by spontaneous mutation or by serial extractions of S+ cells with water. Reattachment occurred in the presence of divalent (Ba2+, Ca2+, Co2+, and Mg2+) but not monovalent (H+, NH4+, Na+, K+) or trivalent (Fe3+) cations. The 98-, 125-, 127-, and 149-kDa S-layer proteins isolated from strains containing type A LPS (type A ***S*** - ***layer*** ***protein***) all reattached to S- template cells containing type A LPS (type A cells) but not to type B cells. The 98-kDa type B ***S*** - ***layer*** ***protein*** reattached to SAP- type B cells but not to type A cells. ***Recombinant*** 98-kDa type A ***S*** - ***layer*** ***protein*** and its truncated amino-terminal 65- and 50-kDa segments expressed in Escherichia coli retained the full and specific determinants for attachment. ***S*** - ***layer*** ***protein*** and purified homologous but not heterologous LPS in the presence of calcium produced insoluble complexes. By quantitative enzyme-linked immunosorbent assay, the ***S*** - ***layer*** ***protein*** copy number per C. fetus cell was determined to be approximately 10(5). In conclusion, C. fetus cells are encapsulated by a large number of ***S*** - ***layer*** ***protein*** molecules which may be specifically attached through the N-terminal half of the molecule to LPS in the presence of divalent cations.

L33 ANSWER 65 OF 66 MEDLINE
AN 89327128 MEDLINE
DN 89327128 PubMed ID: 2666389
TI Cloning and sequencing of the gene encoding a 125-kilodalton surface-layer protein from Bacillus sphaericus 2362 and of a related cryptic gene.
AU Bowditch R D; Baumann P; Yousten A A
CS Department of Microbiology, University of California, Davis 95616.
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AB Using the vector pGEM-4-blue, a 4,251-base-pair DNA fragment containing the gene for the surface (***S***)- ***layer*** ***protein*** of Bacillus sphaericus 2362 was cloned into Escherichia coli. Determination of the nucleotide sequence indicated an open reading frame (ORF) coding for a protein of 1,176 amino acids with a molecular size of 125 kilodaltons (kDa). A protein of this size which reacted with antibody to the 122-kDa ***S*** - ***layer*** ***protein*** of B. sphaericus was detected in cells of E. coli containing the ***recombinant*** plasmid. Analysis of the deduced amino acid sequence indicated a highly hydrophobic N-terminal region which had the characteristics of a leader peptide. The first amino acid of the N-terminal sequence of the 122-kDa ***S*** - ***layer*** ***protein*** followed the predicted cleavage site of the leader peptide in the 125-kDa protein. A sequence characteristic of promoters expressed during vegetative growth was found within a 177-base-pair region upstream from the ORF coding for the 125-kDa protein. This putative promoter may account for the expression of this gene during the vegetative growth of B. sphaericus and E. coli. The gene for the 125-kDa protein was followed by an inverted repeat characteristic of terminators. Downstream from this gene (11.2 kilobases) was an ORF coding for a putative 80-kDa protein having a high sequence similarity to the 125-kDa protein. Evidence was presented indicating that this gene is cryptic.

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TI IV. Molecular biology of S-layers
AU Bahl, H.; Scholz, H.; Bayan, N.*; Chami, M.; Leblon, G.; Gulik-Krzywicki, T.; Shechter, E.; Fouet, A.; Mesnage, S.; Tosi-Couture, E.; Gounon, P.; Mock, M.; De Macario, E.C.; Macario, A.J.L.; Fernandez-Herrero, L.A.; et al.
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AB In this chapter we report on the molecular biology of crystalline surface layers of different ***bacterial*** groups. The limited information indicates that there are many variations on a common theme. Sequence variety, antigenic diversity, gene expression, rearrangements, influence

of environmental factors and applied aspects are addressed. There is considerable variety in the S-layer composition, which was elucidated by sequence analysis of the corresponding genes. In Corynebacterium glutamicum one major cell wall protein is responsible for the formation of a highly ordered, hexagonal array. In contrast, two abundant surface proteins form the S-layer of Bacillus anthracis. Each protein possesses three S-layer homology motifs and one protein could be a virulence factor. The antigenic diversity and ABC transporters are important features, which have been studied in methanogenic archaea. The expression of the S-layer components is controlled by three genes in the case of Thermus thermophilus. One has repressor activity on the S-layer gene promoter, the second codes for the ***S*** - ***layer*** ***protein*** . The rearrangement by reciprocal recombination was investigated in Campylobacter fetus. 7-8 S-layer proteins with a high degree of homology at the 5' and 3' ends were found. Environmental changes influence the surface properties of Bacillus stearothermophilus. Depending on oxygen supply, this species produces different S-layer proteins. Finally, the molecular bases for some applications are discussed. ***Recombinant*** S-layer ***fusion*** proteins have been designed for biotechnology.